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THE ACTIVITY OF THE PACEMAKER PREVIOUS TO THE DISCHARGE OF A MUSCULAR IMPULSE[1]

EMIL BOZLER

From the Department of Physiology, the Ohio State University, Columbus

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In the present paper it will be shown for the ureter and the intestine that a characteristic weak activity always precedes, and apparently initiates, the spontaneous discharge of an all or none conducted impulse. This non-conducted activity consists either in a gradually increasing negativity or in rhythmic potential variations. In the ureter, furthermore, a slight rise in tonus, similar in its time relations to the electric change, precedes each spontaneous contraction.

The initiation of spontaneous activity in muscle was previously studied only in the heart. Adrian (1) and Eccles and Hoff (16) have failed to detect electric changes preceding the discharge of an impulse in the pacemaker of the vertebrate heart. In the heart of a snail Arvanitaki (4), however, clearly demonstrated slow changes in electric potential and in tonus previous to each beat. His results agree in their fundamental aspects with my own observations on vertebrate smooth muscle. Recently, similar results were also obtained for vertebrate cardiac muscle (15). The similarity of the phenomena in these histologically so different muscles derived from different kinds of organisms is significant because it suggests that the processes underlying automaticity are essentially the same in all muscles.

The artificial initiation of rhythmic activity in skeletal muscle by chemical substances and by constant electric current closely resembles normal automaticity. It is interesting, therefore, that the discharge of impulses in these tissues (for skeletal muscle cf. 2, 5; for nerve cf. 4) is initiated by local potentials which are similar to those which will be described here for the normal activity of smooth muscle and which have also been found in vertebrate cardiac muscle (15). Recent studies, furthermore, have shown that a local reaction also precedes the discharge of an impulse produced by electric shocks and by motor nerve impulses. It appears probable, therefore, that all conducted responses of nerve and muscle, whether spontaneous or elicited by stimuli are initiated by

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local graded reactions which are characterized electrically by a gradual rise in negativity.

METHODS. The isolated ureter of the dog, cat and guinea pig, and the small intestine of the guinea pig were used. The ureter was dissected up to the renal pelvis and carefully freed of extraneous tissue.

In the ureter sometimes spontaneous activity did not begin until several hours after the preparation was set up and then contractions occurred infrequently. Asphyxia during the dissection seems to have been the chief cause of this difficulty. Rapid dissection and the use of chilled and aerated Ringer's solution improved the results. Furthermore, it was found that motility was less in Ringer's solution made up in copper distilled water than in solutions prepared with glass distilled water. The exact composition of the Ringer's solution seems to be of less importance. Preliminary experiments showed that the action of calcium and potassium ions on the ureter is essentially like that on the heart. A moderate increase in calcium increases the duration and strength of the contraction without appreciably influencing the rate; increase in potassium has the opposite effect. The pH has no appreciable effect within a wide range. Ringer-Locke solution buffered with phosphate was found nearly optimal for the experiments.

The technique of recording potentials was the same as that described in the following paper. Because the changes studied were slow, a mechanical recorder writing on a smoked drum was ordinarily used (natural frequency 40 per sec.). A comparison of the graphs with those obtained with an oscillograph showed that no distortion was introduced by the recorder. It should be noted, however, that the records do not correctly indicate the amplitude of the conducted responses because the latter are usually more than fifty times stronger than the local activity and, consequently, are largely cut off by the characteristics of the amplifier.

The results which will be reported here show that local potentials may occur simultaneously over a wide region of a muscle. Since the records merely indicate the difference in the activity at the two leads these potentials may escape attention if both leads are on an active region. To avoid this possibility it would be desirable to place one lead on an injured region so as to obtain monophasic potentials. However, serious difficulties are encountered in recording monophasic potentials in this manner for more than a few minutes (cf. 14) and, furthermore, complications are introduced by the injury itself because the border of the injured region may temporarily act as a pacemaker. The conditions under which these difficulties were avoided will be described later.

In the ureter the origin of the impulses was determined by placing the leads close together and observing which of the leads became negative first. This procedure was repeated, shifting the leads in the direction from which the impulses were coming. At the origin of the impulses the diphasic action potentials begin to become inverted.

In one series of experiments the contractions of the ureter were recorded by a very sensitive isometric lever. The preparations were immersed in Ringer's solution.

RESULTS. *Ureter.* This organ is unusually favorable for the study of the activity of the pacemaker because normally discharges occur at rather long intervals and originate in a distinct region, the extreme renal end. There is a gradient of diminishing automaticity away from this region as indicated by the fact that after cutting the organ the caudal part discharges at a slower rate than the renal region. The middle region alone, if suspended in air, discharges only rarely.

At the beginning of an experiment, as soon as spontaneous activity begins, the

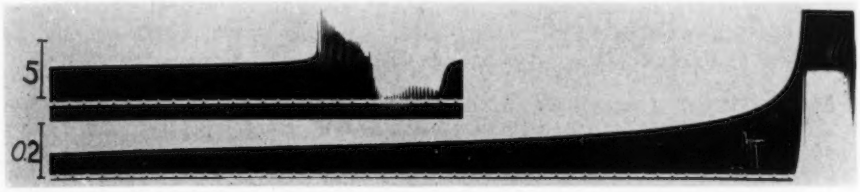

Fig. 1. Slow rise in negativity of the pacemaker preceding a spontaneous peristaltic wave in the ureter of the guinea pig, recorded by oscillograph. First lead on pacemaker, second 2.4 cm. distant. Both records from the same preparation at different amplification. On left calibration in mVolt. Temp. 36°. Time $\frac{1}{5}$ sec.

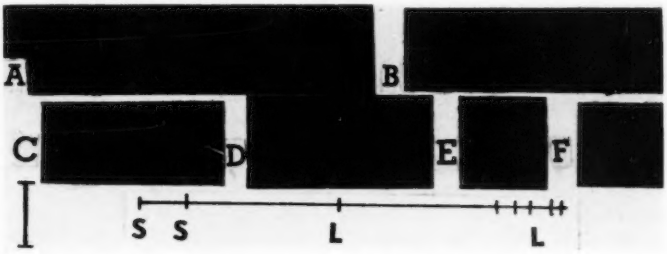

Fig. 2. Records showing magnitude of local potentials at different distances from pacemaker of the cat's ureter. First lead: in A on pacemaker, in B 3 mm., in C 5 mm., in D 7 mm., in E 1 cm. closer to second lead. F: contraction produced by electric shock and conducted antidromically, first downstroke shock artifact. Below: diagram of arrangement of electrodes. S: stimulating electrodes. L: leads to amplifier. Calibration: one mVolt. Temp. 36°. Time, seconds.

origin of the impulses was determined as described above. It was usually found near the renal end of the organ. If one lead was placed on the renal end, the other 2 or more cm. caudad, every peristaltic contraction was preceded by slow potential changes which occur in two forms: 1, a gradually increasing negativity of the renal end, the rise becoming progressively faster until the moment of discharge of the conducted response (fig. 1, 2); 2, potential oscillations which gradually increase in magnitude until a discharge occurs (fig. 3). If rhythmic potentials are present, the impulses are discharged near a crest and at a time when the renal end is negative.

It can be shown that the potentials preceding a spontaneous discharge are produced in the region where the conducted responses originate, usually at the renal end of the ureter. They are largest if one lead is near the renal end of the ureter, they diminish rapidly caudad and are practically absent if the lead is more than 1 cm. from the renal end of the organ (fig. 2). A change in the position of the other lead has no influence on the magnitude of the slow potentials provided it is at least 2 cm. away from the renal end. In this connection it should be remembered that electronic spread of potentials is insignificant in smooth muscle. The distribution of the potential variations near the pacemaker, therefore, correctly represents the processes in the region of the leads.

In exceptional cases impulses also have originated in the middle or caudal region of the ureter but usually only for short periods of time. In this case

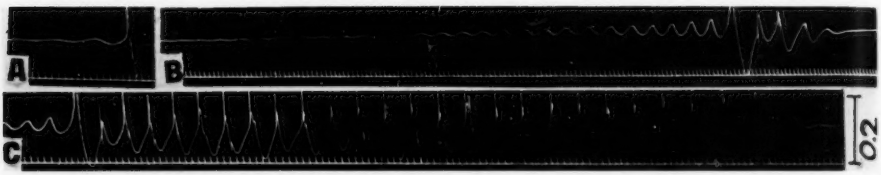

Fig. 3. Oscillatory local potentials from ureter of guinea pig. First lead on pacemaker. A: single discharge. B: long series of oscillations in depressed preparation. C: a group of discharges. Calibration: 0.2 mVolt. Temp. 34°. Time, seconds.

Fig. 4. Effect of premature contractions on rhythmicity of the cat's ureter. First lead on pacemaker. Two premature peristaltic waves, produced by electric shock and conducted antidromically. Arrangement of electrodes as in figure 2. Downstroke preceding premature contraction shock artifact. Calibration: 1 mVolt. Temp. 36°. Time, seconds.

the characteristic local potentials were observed near the place of origin, whereas the renal end appeared silent.

The observations just mentioned show that the slow potentials have nothing to do with the conduction of impulses. This is also demonstrated by the fact that slow potentials are absent in responses induced by an electric shock (fig. 2F, 4). It can be concluded, therefore, that these potentials are the expression of a non-conducted activity of the pacemaker preceding the discharge of impulse. They may be interpreted as a slow depolarization of the cell surface.

The magnitude of the slow potentials up to the moment when the discharge occurs is usually only about one-fiftieth of the magnitude of the conducted response. The possibility that these weak electric changes are artifacts is improbable because in preparations mounted in a moist chamber only drastic movements produce detectable potential changes and because the local potentials are not accompanied by visible movements.

To determine whether the local potentials are accompanied by a contraction, the tension of the muscle was recorded. A short piece, about 1 cm. long, from

the renal portion of the dog's ureter was used. Previous to each spontaneous contraction there was a gradual rise in tonus or a brief series of tonus waves (fig. 5). In agreement with the small size of the local potentials the mechanical changes were very weak. The tension developed in the ureter of a dog of moderate to large size never exceeded 40 mgm., only about one-fiftieth of the tension produced in a peristaltic contraction. The rhythmic variations in tonus were not observed as clearly as the corresponding potential changes, partly because of the smaller sensitivity of the tension lever as compared with the electric recording system; partly also, perhaps, because of some incoördination between different parts of the preparation which tends to smooth out the oscillations.

The magnitude of the tonus changes varied greatly under different conditions. It was greatest if the organ showed great motility. In muscles where spontaneous beats occurred at very long and irregular intervals sometimes the increase in tonus before a contraction was not detectable. Such cases can be explained by assuming that a very small portion of the muscle was involved in the initiation of a contraction. It seems probable that the depolarization

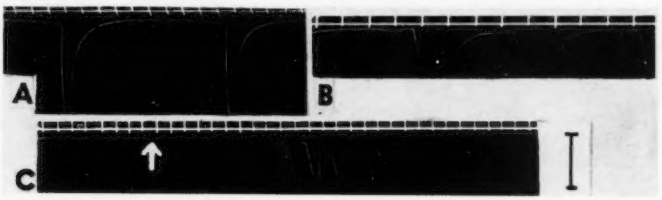

Fig. 5. Tension records from ureter of dog showing gradual rise in tonus (A) and tonus waves (B) before a spontaneous contraction. Preparation 1 cm. long, taken from the renal region. C, taken before spontaneous activity started, shows rise in tonus after adding 1 drop of adrenaline 1:1000 to bath (indicated by arrow). Calibration: 0.2 gram. Temp. 37°. Time, 5 sec.

of a large region, as normally occurs, is unnecessary for the initiation of an impulse. Occasional negative findings, therefore, do not invalidate the positive results obtained in the more active preparations.

A tonus increase also invariably precedes the response elicited by adrenaline (fig. 5C) and by stretching (15).

Small intestine of the guinea pig. The study of the initiation of spontaneous contractions in the intestine presents much greater difficulties than in the ureter because bursts of impulses may be discharged simultaneously from many regions of the organ and because the origin of the discharge varies almost continuously. Unfortunately, there is no entirely satisfactory method of recording simultaneously local potentials and conducted impulses. If the leads are widely separated, as was the case in most of the work of previous investigators, the action potentials of conducted impulses are greatly distorted and difficult to recognize (cf. 11). If the leads are close together, as in my earlier studies (11), the discharge of impulses can be recorded clearly, but local potentials which involve appreciable parts of the muscle are masked.

However, an approach to a monophasic potential without mechanical injury

was obtained by drawing one end of the preparation into a narrow glass tube and attaching one lead to this part of the muscle. The region inside the tube became asphyxiated and inactive. The inactivity under one lead greatly simplified the records. Purely monophasic potentials were usually not obtained, probably because the active lead was too far removed from the inactive region (cf. 14).

The records obtained under the conditions just described show two distinct types of potential changes. While the muscle appears quiescent there are fairly regular smooth rhythmic potential variations at a frequency of about one every 1.2 seconds. In addition there are bursts of impulses which accompany the peristaltic waves and have previously been described (11). Usually the peristaltic waves start out from the same region repeatedly. If the active lead happens to be on such a region the impulses rise from the negative crest of some of the

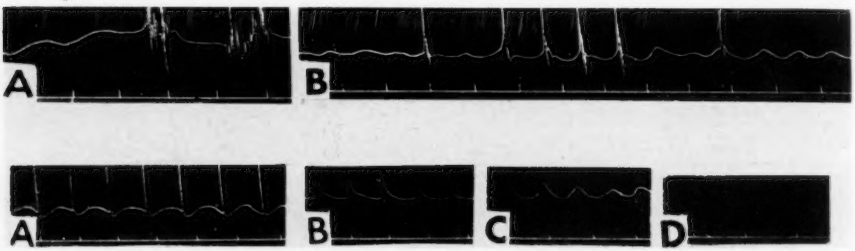

Fig. 6. (top) Potentials from the small intestine of the guinea pig showing local potentials and impulses. One lead on part of the muscle inside glass tube. A: fresh preparation, peristalsis. B: obtained about 30 minutes after A. Temp. 34°. Time, seconds.

Fig. 7. (bottom) Potentials from a contraction ring of the small intestine of the guinea pig. Preparation was quiescent except for slight contraction waves in contracted ring. A: active lead in center of ring, B: 2 mm., C: 4 mm., from center. D: lead outside ring. Temp. 34°. Time, seconds.

slow waves. A shift of the pacemaker occurred frequently and was often indicated in the potential record (fig. 6A).

The relation between the slow potentials and the discharge of impulses could be observed more clearly one half to one hour after the preparations had been brought into the moist chamber, and after they had become less active. Their activity then resembled pendular movements. Single impulses or small bursts of impulses were discharged at a fairly regular frequency. These impulses also arose from the negative crest of the potential waves (fig. 6B).

Shortly before the activity of a preparation ceased permanently, contraction rings often remained at the same place for long periods of time while movements were slight or absent. Strong rhythmic potential variations occurred in this region. At the place of strongest contraction impulses were discharged (fig. 7). On both sides there was a region where the impulses were very weak and still farther away there were only slow potential waves without any discharge of impulses. Such slow waves, however weaker and less regular, were also found

on the rest of the intestine which appeared inactive. It seems that impulses were discharged from a point in the central part of the contraction ring and that they were conducted decrementally in both directions for a few millimeters.

The slow potential variations just described probably are identical with those which have been recorded by previous observers in the intestine of the rabbit (3, 8, 9, 10) and dog (17). They have been demonstrated most convincingly by Berkson who observed that they continue almost unchanged after movements have been stopped by adrenaline, atropine and other drugs. I have confirmed this result for the intestine of the guinea pig. Balassa (6) reported a similar observation for the uterus of the cat. As pointed out by Berkson (9), these facts show clearly that the slow potential waves are not the action potentials of the rhythmic contractions of the intestine. That these contractions are due to the discharge of conducted impulses is shown by the fact that they are accompanied by a regular series of spike potentials (11).

An explanation for the slow potentials is suggested by the observation that each discharge is preceded by a phase of rising negativity. It appears, therefore, that the slow potentials are analogous to those of the other muscles studied and represent local potentials which initiate impulses. These potentials can be regarded as an expression of the automaticity of the muscle. Whether they actually produce impulses naturally depends also on whether muscular excitability is high enough for the conduction of impulses. It appears plausible, therefore, that the slow potentials may continue while no contractions occur, as is the case under the influence of adrenaline and as it has also been observed in the ureter and in cardiac muscle (15) during a state of low excitability. Berkson's suggestion that the slow potentials are a regulating influence on the muscular rhythm, although not originating it, agrees in part with the explanation offered here, but his assumption that the potentials are caused by the action of the intrinsic nervous plexus appears unnecessary.

The action of some of the other drugs studied might seem to contradict the explanation offered here. Nicotine and curare were found to diminish or abolish the local potentials while the contractions became less frequent and often stronger (10). This result can be explained by assuming that the drugs diminish the tendency of the cell surface to depolarize spontaneously. In this case it is possible that the local potentials originating the contractions are produced only in small regions of the muscle and, therefore, appear smaller or may escape attention.

DISCUSSION. 1. *The nature of the local potentials and their relation to automaticity.* The observations reported here suggest that a spontaneous, non-conducted activity, indicated by a change in electric potential and of tonus, is the basis of the automaticity of visceral muscles. The local potentials differ from impulses not only by the absence of conduction but also by their slowness. The assumption that the underlying process is an asynchronous discharge of impulses conducted for short distances is unlikely in view of the smoothness and regularity of the process. The slow potentials, therefore, can best be interpreted as a non-conducted depolarization of the cell surface. This interpretation

agrees with that which has been given to similar potentials in nerve fibers and skeletal muscle during the artificial initiation of impulses by chemical substances.

The tendency of the cell surface to depolarize spontaneously is not restricted to the exact region where the impulses originate. Thus, in the intestine, the local potentials can be found in all regions of the muscle, in agreement with the fact that the degree of automaticity is fairly uniform throughout the intestine. In the ureter the local potentials can usually be recorded from a region which is about 1 cm. long. However, the activity is strongest at the exact place where the impulses are initiated and there is a gradient of local activity away from this region (fig. 2). This gradient agrees with that of automaticity.

To avoid misunderstanding it must be emphasized that not all slow potential changes in muscle are local potentials like those which have been described here. In cardiac muscle and in some smooth muscles there are in addition states of negativity which have been called residual negativity (14). These potentials differ from the local potentials by the fact that they always follow the process of conduction and that they are about as large as the spike potentials. Furthermore, slow potential changes may be recorded which are simply due to the fusion of spike potentials resulting from an unfavorable arrangement of the leads (14).

2. *The significance of the local potentials for the rhythmicity of smooth muscle.* In the ureter spontaneous contractions often follow each other at regular intervals of 15 seconds or longer. In this case each response is initiated by a slow rise in negativity. The conducted response produces repolarization, thereby restoring the previous condition of the pacemaker (fig. 4). Repolarization also follows the response to a stimulus. This observation explains why the pause following a premature response is as great as a normal pause, in agreement with the well-known result of similar experiments on the heart.

The contractions of the ureter may also occur in groups, separated by several minutes of complete rest. The frequency of the beats within one group is high, one beat every 3 to 4 seconds. This type of activity is always associated with the presence of oscillatory local potentials and it is due to the fact that several oscillations in succession set up conducted responses. At the end of each group there are further oscillations of gradually diminishing magnitude. The Luciani groups of cardiac muscle closely resemble the phenomenon just described. They are also due to the presence of oscillatory local potentials (15).

The grouping of responses in the ureter and the oscillatory local potentials probably are abnormal phenomena. Oscillations were found most frequently in preparations which had been kept in Ringer's solution for some hours before the observation. As a preparation deteriorates, the number of oscillations before the discharge increases (fig. 3B). There may also be oscillations of gradually increasing and decreasing magnitude which do not produce any conducted impulses.

3. *Inhibition in visceral muscles.* It is surprising that the local potential oscillations are largely independent from variations in muscular excitability.

This is best demonstrated by the action of adrenaline on the intestine. The drug greatly diminishes excitability and stops the conduction of impulses (12), but it does not significantly alter the local potentials. It has, furthermore, been shown that potential oscillations may continue in deteriorated preparations with undiminished intensity and frequency while no impulses are discharged, a condition indicating low excitability.

The observations just mentioned have an important bearing on the problem of inhibition in visceral smooth muscle. It has been concluded (12) from an entirely different series of observations that inhibition is entirely due to the experimentally demonstrated decrease in muscular excitability and to the consequent diminution or cessation of the discharge of impulses and not due to any decrease in the intrinsic stimulus for the discharge of impulses. This conclusion is confirmed by the fact that the local potentials are not abolished during inhibition. To explain the transitory excitatory action of adrenaline preceding the inhibition, as observed in the uterus of many species, it was assumed, furthermore, that adrenaline, besides decreasing excitability, causes an increase in the tendency for setting up impulses in uterine muscle. This assumption is confirmed by the observation of Balassa and Gurd (7) that adrenaline, although it inhibits motility, actually increases the size of the local potentials in the cat's uterus.

4. *Local potentials and tonus.* The results reported here are significant for the question of the tonus of smooth muscle. The absence of spike potentials indicates that the slowly developing weak contraction of the ureter previous to each spontaneous peristalsis is not a tetanic contraction. However, the difference between this continuous type of contraction and that due to conducted impulses probably lies only in the underlying excitatory mechanism, not in the mechanism of contraction.

Because the term tonus generally is used purely descriptively for any weak, sustained contraction, it should not be expected that the underlying mechanism is the same in all cases. It has been shown (13) that tonic contractions of visceral smooth muscles are often accompanied by a discharge of conducted impulses and are, therefore, not essentially different from tetanic contractions. It is not possible to distinguish this type of contraction from non-propagated activity except by a careful analysis of the accompanying electric changes.

It is probable that the tonus of visceral smooth muscles generally is predominantly due to a tetanic contraction because, in those cases which have been studied as yet, local activity, not accompanied by a discharge of impulses, is so weak that it does not produce any visible movements. It seems that the latter type of contraction has no significance for the motility of the muscle and is merely incidental to the membrane changes which are responsible for the initiation of muscular impulses. The close correlation between mechanical and electrical changes in the absence of conduction, as found in the ureter, presents an interesting, but as yet obscure, problem. This phenomenon may be taken as an indication that the ultimate causes of automaticity are fluctuations in metabolism.

SUMMARY

The action potentials accompanying spontaneous contractions were studied in the ureter and the intestine. At the pacemaker each conducted impulse is preceded by a gradual rise in negativity. This electric change, which is much smaller than the spike potential, is non-conducted. In the ureter it is found only within a region of about 1 cm. from the origin of the impulses and it is strongest at the pacemaker itself. The local potentials can be interpreted as changes in the polarization of the cell surface. They can be considered as the basis of the automaticity of smooth muscle.

In the ureter the local potentials are either a gradually rising negativity or oscillatory changes of gradually increasing amplitude. In the latter case the impulses arise from the negative crest of some of the oscillations and they are often discharged in groups. The former type of local potential must be considered as the normal type because it is generally found in preparations in which the spontaneous motility is like that under normal conditions.

In the intestine slow potential waves are almost continuously present in all regions of the organ. Impulses are discharged, often in bursts, at the negative crest of such waves. The evidence indicates that the slow potentials are due to a non-conducted activity which has the function of initiating impulses.

In all the tissues studied local potential changes can take place while no impulses are discharged. This condition is produced by a depression of the excitability of the muscle, in the intestine, for instance, by adrenaline. The implications of this independence of local potentials from excitability for the question of the inhibition of smooth muscle are discussed.

The local potentials are not accompanied by visible movements of the muscle but in the ureter a small rise in tension, running closely parallel with the local potential, precedes each spontaneous contraction. Also, the contractions elicited by adrenaline are preceded by a slow rise in tonus.

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THE ACTION POTENTIALS ACCOMPANYING CONDUCTED RESPONSES IN VISCERAL SMOOTH MUSCLES[1]

EMIL BOZLER

From the Department of Physiology, Ohio State University, Columbus

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It has been shown previously that a wave of contraction in visceral smooth muscles is accompanied by an action potential which usually consists of brief spikes similar to those of nerve and striated muscle, but also potential changes of longer duration like those of cardiac muscle were observed (5, 7). In every case the action potentials consisted of waves of negativity suggesting that the mechanism of conduction in smooth muscle is essentially the same as in other excitable tissues. The experiments reported here confirm these results. Furthermore, the slow phases of the action potentials, which may be large and of long duration, but could not be recorded satisfactorily by the apparatus previously used, were studied by means of a direct coupled amplifier.

The electric changes in smooth muscle as well as in any other tissue can be interpreted only under favorable experimental conditions. For this reason the precise meaning of many records of action potentials of smooth muscle reported in the literature remains uncertain. Most of these records show irregular oscillations superimposed on slow variations of the baseline. It must be emphasized that such slow potentials do not prove the existence of slow electric changes which are different and independent from spike potentials, even if the possibility of movement artifacts has been eliminated. Such potentials can be produced by the incomplete resolution of spikes which occurs if the effective width of the leads is greater than the distance between two impulses traveling along the muscle. The resulting fusion will become the more complete the greater the width and distance of the leads and the thicker the mass of tissue between the leads. This interpretation can be applied to the recent work of Bourdillon (3) and Bourdillon and Lidwell (4) on the action potentials of the rabbit's vagina. Whether the slow potential variations observed in this organ are due to a process different from spike potentials, as the authors suggest, cannot be decided without further analysis.

In comparing the action potentials of smooth muscle with those of nerve and skeletal muscle it is important to keep in mind the different dimensions in these types of tissues. In smooth muscle the width of the active region in a spike potential is less than 1 mm. Thus, during a peristaltic wave of the guinea pig's ureter the spikes follow each other at a distance of less than 1 mm. (7). It may be said, therefore, that 1 mm. in smooth muscle is equivalent to a distance of at least a few centimeters in nerve or skeletal muscle. This difference is explained

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I am indebted to Dr. H. Grundfest for valuable advice in the construction of the amplifier used.

by the small diameter of the fibers of smooth muscle. For the same reason electrotonic spread of potentials is slight as compared with nerve.

METHODS. Uterine strips from cats and guinea pigs during estrus and pregnancy, strips from the small intestine of the rabbit and cat, and the ureter of the cat, dog, rabbit, guinea pig and rat were used. In the cat, estrus was produced by injecting theelin (500 int. units) every two days for four (incomplete estrus) or five (complete estrus) days.

The muscles were prepared and mounted essentially as described previously (5). They were left in Ringer's solution for one-half to one hour before the start of the experiment. To prevent drying it was found advantageous, if very thin muscles were used, to wet the walls of the chamber with water or dilute Ringer's solution.

Very small calomel electrodes were used as leads. They made contact with the muscle by flexible thin cottonwicks (less than 1 mm. in width at the muscle), soaked in Ringer's solution. The potential changes were recorded by a direct coupled Thoenis amplifier and a G. E. oscillograph. The grid leads of the input were 100 megohm. Preliminary records were usually taken by a mechanical recorder on a smoked drum. If the preparations were carefully prepared and the surrounding tissue was completely removed the spike potentials generally had a magnitude of 4 to 6 mVolt.

The electric stimuli were condenser discharges (7_{μ}F) applied through platinum electrodes. Ringer-Locke's solution buffered by phosphate was used (0.9 per cent NaCl, 0.024 per cent $CaCl_2$, 0.042 per cent KCl). A solution of rather high Ca-ion concentration is essential for maintaining excitability and for obtaining strong contractions.

RESULTS. *Interpretation of monophasic and diphasic potentials.* Monophasic action potentials were obtained if the distant lead was on an inactive region of the muscle. This was usually accomplished by pinching the preparation between the leads. The potentials were partly diphasic unless they were led off from a point less than 1 mm. from the injured region. That this distance has to be much shorter than in nerve is to be expected on the basis of the electric conditions in the tissue (cf. 1, 10) if it is considered that the length of the active region during the conduction of an impulse is usually less than 1 mm.

As a general practice the proximal lead was placed directly on the margin of the injured region. If the lead was shifted so that it partly covered this region, the potentials did not change their character but were weaker. If the lead was placed on intact tissue more than 1 mm. away from the injury the potentials became somewhat greater, but had a rapid downstroke and subsequent hump in the falling phase, or a brief positive wave following the spike. These variations were considered as diphasic artifacts and resemble corresponding phenomena in nerve. The potentials remained strictly monophasic for only a short time. After a few minutes they gradually became more and more diphasic. For this reason, all the monophasic records described here were obtained within 2 minutes after injury.

The monophasic potentials show a great diversity. They may consist of

single brief spikes (fig. 1C), a repetitive discharge of such spikes (fig. 3C), or a long-lasting negativity (fig. 2D). In view of the necessity of placing the leads

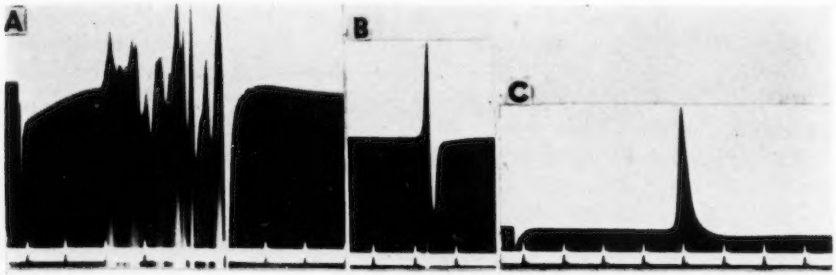

Fig. 1. Potentials from a uterine strip of the cat, an example of a brief impulse. The cat was in incomplete estrus. A: diphasic with long distance (2.2 cm.) between leads. Distance between stimulating cathode and first lead 1.5 cm. The last downstroke is due to the passage of the impulse across the second lead and should be compared with the monophasic potential. First downstroke stimulus artifact. B: Diphasic with short distance (1 mm.) between leads. Note absence of T-wave. C: monophasic. Temp. 34°. Time $\frac{1}{5}$ sec.

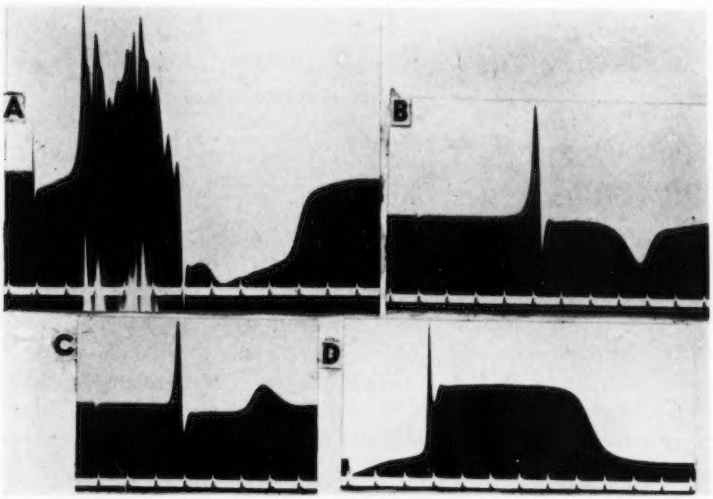

Fig. 2. Potentials from the ureter of the rat showing a long continued state of negativity. A: Diphasic potential with long distance between leads (2.1 cm.). The rapid downstroke in the middle of the record indicates the arrival of the impulse at the second lead. B: Diphasic with short distance (1 mm.) between leads, showing R- and T-waves. C: The same but from other region. Positive T-wave. D: Monophasic. Temp. 34°. Time $\frac{1}{5}$ sec.

very close to the injury, the possibility that these monophasic action potentials are abnormal must be examined. Diphasic records are useful for this purpose.

Because of the shortness of the active region it is usually possible to separate the electric changes occurring at the two leads. With widely separated leads, it may be expected that the positive wave of the diphasic potential is a relatively undistorted record of the potential change at the distal lead and that it should resemble the monophasic potential, because an impulse cannot have any noticeable effect after it has passed beyond the leads. In agreement with theoretical expectation this last phase of the diphasic potential is roughly the mirror image of the monophasic potential for a given preparation, as shown in figures 1, 2 and 3.

The first part of the diphasic potential obtained with widely separated leads consists of a rapid upstroke followed by highly irregular, but exactly reproducible, potential variations. That these irregularities are due to local conditions along the path of conduction is shown by the previous observation that the various peaks of the potential record can be eliminated one by one by blocking con-

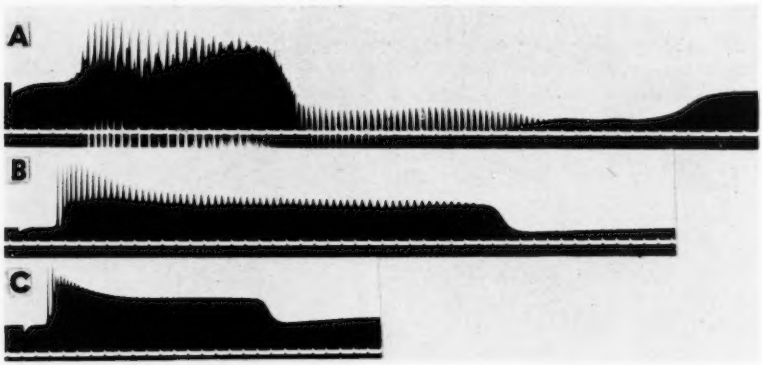

Fig. 3. Potentials from ureter of guinea pig showing a repetitive response of high frequency and residual negativity at the end of the discharge. A: Diphasic with long distance between leads (2.8 cm.). B: Monophasic from same preparation as A. C: Monophasic from another preparation. Temp. 30°. Time $\frac{1}{5}$ sec.

duction at various places between the leads, progressing from the distal to the proximal lead (5). This result was confirmed by placing the distal lead gradually closer to the proximal lead. Under these conditions the positive phase of the diphasic potential remained unchanged but the first, irregular, phase became progressively shorter.

To explain the diphasic potential, it has been suggested (9) that the smooth muscle preparations behave like a series of muscular units, a condition which could arise from the varying width of the anastomoses between the muscle fibers. However, one would expect, then, that the separation into units also manifests itself in other respects. For instance an electric current should produce polarization not only close to the electrodes but also at regions where the anastomoses are very narrow. On the contrary it was found that electric current stimulated at only one electrode, even if the stimulus was many times above threshold.

It is more probable that the irregular variations accompanying the conduction

of the impulse between the leads is caused by local asymmetries of the preparation, due to attached extraneous tissue, local injury, etc. That such conditions can produce phenomena like those described here has been shown for nerve (cf. 1, 10) but the effects are even more conspicuous in smooth muscle because the distance between the leads was very large as compared with the length of the active region.

Comparison of the action potentials of different muscles. The rising phase is brief in all preparations. The peak is reached in 0.05 to 0.1 second. These values, however, do not indicate the true time relations of the potential change, because most or all of this time interval is accounted for by the conduction over the effective width of the lead. The fact that the frequency of discharge is as high as 40 impulses per second in one of the preparations used indicates that the duration of the spike process alone is less than 25 msec. and it may possibly have the same order of magnitude as in nerve.

The total duration of the action potential, on the other hand, may be long and it varies within wide limits in different muscles. It was shortest in the uterus of the cat during incomplete estrus (fig. 1), and in the small intestine. The potential has a brief spike, but the return to the baseline is slow. It is similar in its time relations to that of skeletal muscle (cf. Bishop, Young).

The other extreme is the ureter of the rat (fig. 2). After an initial spike a considerable negativity is maintained at a fairly constant level for various periods of time up to 10 seconds. The duration seems to depend largely on excitability. Cocain, which raises electric excitability, particularly in depressed preparations, prolongs the action potential. The similarity with the action potential of cardiac muscle is striking. In diphasic potentials, a T-wave, often positive, is present. If the distance between the leads is varied the duration of the R-wave varies proportionately, but the R–T interval remains unchanged. The same type of action potential, but somewhat shorter in duration, was observed in the ureter of all the other species studied (cat, dog, rabbit), with the exception of the guinea pig.

The initial spike of the monophasic potential of the ureter probably is not a diphasic artifact because it is always present in the positive wave of the diphasic potential (fig. 2). In monophasic potentials the spike sometimes was absent and the upstroke slower than normally only if the lead was several millimeters from the block. Pinching the preparation directly below the lead, then, restored the normal character of the potential.

The ureter of the guinea pig (fig. 3) presents intermediate stages between the types of action potential just described. A single stimulus produces a rhythmic discharge of brief spikes, but a state of negativity may persist after the termination of the spike discharge. The frequency may be as high as 40 impulses per second (38°) in highly excitable preparations and it gradually declines during each response. The frequency of discharge is also lower during the refractory phase and under the influence of low calcium Ringer's solution.

The spikes are superimposed on a rising baseline. This phenomenon is partly explained by the fact that the impulses follow each other at a distance of only 1 mm. or less. If the effective width of the lead is greater than this distance

a partial fusion results. However, the fact that a state of negativity often persists at the end of the discharge without any oscillations shows that the rise in the baseline is not entirely due to fusion. An extreme example of this condition is shown in figure 13C, where the rhythmic phase of the action potential appears insignificant. This potential differs only slightly from that of the rat's ureter.

The monophasic action potentials are quite characteristic for the different muscle preparations used. However, in some cases considerable variations were observed. In two out of more than fifty preparations of the rat's ureter, the discharge was repetitive, similar to that of the guinea pig but with a lower frequency of discharge (fig. 4). Furthermore, if the plateau was maintained for several seconds, often the negativity suddenly disappeared and a series of distinct

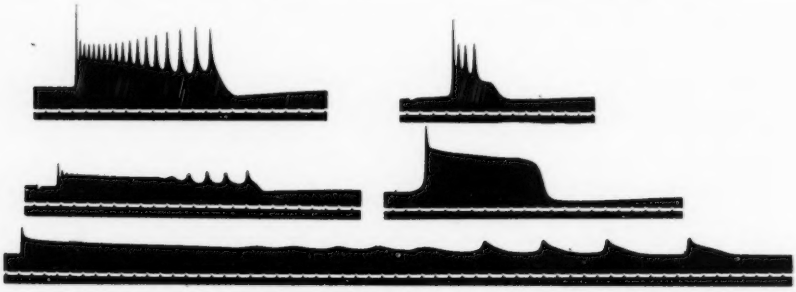

Fig. 4. Monophasic potentials from a preparation of the rat's ureter showing an unusual diversity of electric responses. They were obtained from different regions within 2 hours. Temp. 34°. Time $\frac{1}{5}$ sec.

Fig. 5. Monophasic action potentials from uterine strip of pregnant cat. Discharge elicited by electric shock. The records indicate considerable residual negativity after each wave of activity. Mechanical recorder. Temp. 36°. Time in seconds.

arge spikes were following. Other peculiar rhythmic variations in the action potential of the rat's ureter occasionally observed are shown in figure 4.

Uterine and intestinal strips, although they normally give a repetitive discharge during spontaneous activity (6), usually give only a single brief impulse when a monophasic lead is used, evidently as the result of the injury close to the lead. In strips from the pregnant uterus of the guinea pig, however, a very irregular monophasic potential, beginning with a spike and followed by a high but rather irregular plateau, sometimes was obtained (fig. 5). It is improbable that such potentials occur under normal conditions because diphasic potentials of spontaneous contractions show a fairly regular discharge with a frequency as high as five impulses a second in the uterus and ten impulses per second in the intestine.

A weak positive after potential lasting for 10 to 20 seconds was often, but not invariably, present in the various types of muscle studied.

DISCUSSION. The action potentials of visceral smooth muscles show a much greater diversity than those of other types of muscles. To obtain a unified concept of these potentials two components may be distinguished: a spike potential, which invariably is present in conducted responses; and residual negativity which follows the initial spikes and lasts much longer than the former. The differences between the muscles, then, are considered as due to the prominence of one or the other of these two components. In the action potentials of uterine and intestinal muscle the spike is the most conspicuous feature. The time relations of these potentials resemble closely those of skeletal muscle. In the ureter, however, the spike usually is followed by a high plateau which may be maintained for many seconds. This type of potential resembles closely that of cardiac muscle. There are also intermediate types of potentials showing spikes superimposed on a considerable residual negativity.

The differences in the character of the potentials can be correlated with other physiological differences between the muscles. Uterine and intestinal muscle, which has a weak residual negativity, has a relatively short refractory phase and repetitive stimulation produces a tetanus (5). The ureter, on the other hand, corresponding to the long duration of its potential, has a long refractory phase and does not show summation (5), also in this respect resembling cardiac muscle.

The comparison between different action potentials suggests that the residual negativity, if it is of sufficient magnitude suppresses and takes the place of the spike potentials. It appears probable, therefore, that the state of negativity as observed in the ureter and in cardiac muscle is an excitatory state not essentially different from the spike process.

Records of the isometric contraction of short pieces of the ureter clearly show that the rising phase of the mechanical response lasts much longer than the spike of the action potential. It is probable, therefore, that the slow phase of the action potentials is an accompaniment of the production of mechanical energy as has been suggested by Bishop and Gilson for skeletal muscle. It would not be justified, however, to conclude that the process of contraction itself is giving rise to electric phenomena. At the present state of our knowledge it can only be stated that the degree of polarization of the cell surface probably is closely related to the metabolic changes within the cells. This conclusion is confirmed by the tonus changes which accompany non-conducted potential changes in smooth and cardiac muscle (8).

SUMMARY

Monophasic action potentials accompanying the conducted responses of uterine and intestinal muscle and of the ureter were recorded. The results obtained were confirmed by an analysis of diphasic potentials recorded with widely separated leads.

At the time of arrival of an impulse at the first lead there is a rapid rise in negativity. In other respects the potentials show a great diversity in different

muscles. The monophasic potential is either a brief spike, a long continued negativity or a repetitive discharge. One can group the potentials into a continuous series by distinguishing two components, the spike and residual negativity. The latter component is small in some cases, giving action potentials similar to those of nerve and skeletal muscle. In the ureter of most species, on the other hand, the residual negativity following a single spike is maintained at a considerable magnitude for periods as long as 10 seconds, giving an action potential like that of cardiac muscle. The ureter of the guinea pig is an intermediate type where both components are equally important.

Several facts suggest that residual negativity is a state of activity which is not essentially different from the spike and can take the place of the latter.

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THE ACTIVITY OF THE DESCENDING DUODENUM DURING NAUSEA[1]

FRANZ J. INGELFINGER AND ROBERT E. MOSS

From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts

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Nausea is often accompanied by manifestations of vegetative nervous system activity such as pallor and sweating. That the smooth muscle of the intestines likewise undergoes changes during nausea is a reasonable assumption, especially since nausea and vomiting are such closely related phenomena. To test the validity of this assumption with respect to the duodenum, records of duodenal activity were taken during artificially produced nausea.

PROCEDURE. Subjects for the experiments were 5 normal volunteers and 15 patients. Of the patients, 10 had no organic gastro-intestinal or neurological disease, while in 5 the respective diagnoses were: healed gastric ulcer, minimal hepatitis, syringomyelia, labyrinthitis and myxedema.

The subjects were intubated with the double lumen tube described by Miller and Abbott (1). Each lumen led to one of two tandem balloons which individually held 45 to 50 cc. when filled but not distended with air. In this state, each balloon measured 4.5 to 5.0 cm. in length and 3.5 cm. in diameter. Kymographic records of the intestinal activity were taken with the apparatus used by Ingelfinger and Abbott (2). This apparatus primarily measures volumetric changes in the balloons, since the air in the balloons is kept under more or less constant pressure (10–20 cm. of water).

The two tandem balloons were passed into the descending duodenum under fluoroscopic observation. They were kept in place by a technique which takes advantage of the fact that a single balloon, if inflated proximal to the mid-point of the descending duodenum, is expelled backwards into the stomach; if inflated distal to the mid-point, it is pushed ahead into the transverse duodenum and jejunum (3). Two balloons may therefore be "anchored," provided that they are inflated simultaneously when one balloon lies proximal, the other distal to the midpoint of the descending duodenum (fig. 1). In two cases this technique was not successful, and the tandem balloons had to be "anchored" by inflating the proximal balloon in the gastric antrum.

After a control record of the duodenal activity was taken, the labyrinth was stimulated by gentle irrigation of the external auditory canals with 30 cc. of either cold (18°) or warm (45°) water. Although this method failed to produce nausea in 9 of the 20 subjects, it possesses certain advantages: 1. Nausea, when produced, is very likely of "central" origin. 2. The side reactions of drugs are avoided. 3. The nausea elicited is usually not severe enough to

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provoke the violent muscular contractions of emesis, which interfere with the recording of the intrinsic intestinal activity.

To 5 of the subjects in whom repeated caloric stimulation of the semicircular canals failed to produce nausea, 0.010 gram morphine sulfate was given subcutaneously.

RESULTS. Caloric stimulation of the labyrinth elicited nystagmus and vertigo in all subjects, while varying degrees of nausea were produced 13 times in 11

A B

Fig. 1. Roentgenographic demonstration of the tandem balloons in the descending duodenum. A. The duodenum during a quiet phase. B. Contraction waves compressing the distal part of the proximal balloon and the middle of the distal balloon. These waves are progressing down the intestine.

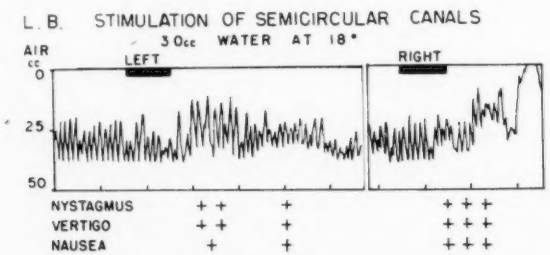

Fig. 2. Record of distal balloon. Time units are minutes. Each of the waves occurring 8 to 9 times per minute is produced by one of the ring-like contractions shown in figure 1, B. After stimulation of the right labyrinth, both the nausea and the duodenal contraction are more marked.

individuals. In 8 of these instances, the descending duodenum underwent a generalized contraction simultaneously with the onset of nausea, the degree and the duration of the contraction being roughly proportional to the intensity and duration of the nausea produced (fig. 2). The usual interval between the beginning of caloric stimulation and the onset of nausea and duodenal spasm

was 1 to 2½ minutes, but in one case these reactions occurred almost immediately. The duodenal contraction, which failed to displace the balloon in 3 instances, persisted for 3½, 6, and 5 minutes respectively. A strong reaction to labyrinthine stimulation was characterized by a complete obliteration of the duodenal lumen (fig. 3), whereas the mildest response observed in these subjects displaced 5 cc. of air from the recording balloon.

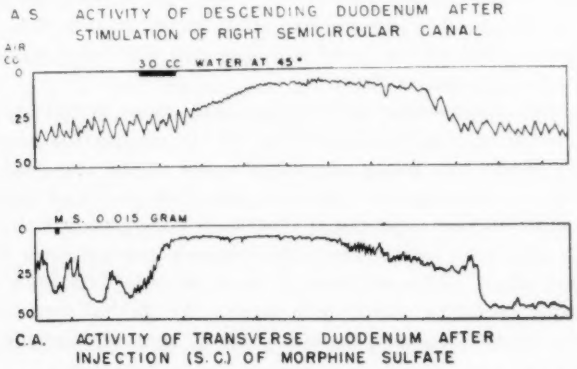

Fig. 3. Records from 2 subjects showing the similarity of a strong duodenal response to caloric excitation of the labyrinth and to the administration of morphine sulfate. Following the contraction induced by morphine, the duodenum is relaxed.

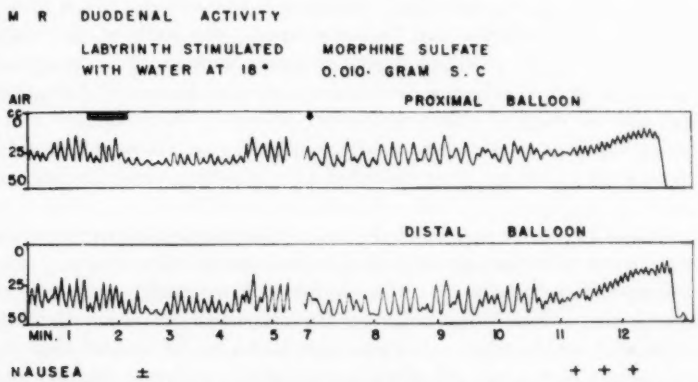

Fig. 4. Simultaneous records of the proximal and the distal balloon. Relaxation of the duodenum during mild nausea following caloric stimulation. Simultaneous contraction of the proximal and distal portions of the descending duodenum following administration of morphine sulfate. Balloons filled with air as they were expelled into stomach.

In 3 subjects, who experienced rather equivocal symptoms of nausea, duodenal activity was decreased in that the duodenum relaxed slightly and the peristaltic waves showed diminished amplitude (fig. 4).

In 2 subjects who vomited after caloric stimulation, the record of duodenal activity was obscured.

Nine subjects, including 3 normal volunteers and 6 patients, failed to evidence nausea after stimulation of the semicircular canals. In these, the record of duodenal activity remained unchanged, even though vertigo was often pronounced.

All the individuals who were given morphine sulfate subsequent to labyrinthine stimulation experienced severe nausea, during which the duodenum contracted strongly. These reactions occurred from $1\frac{1}{2}$ to $3\frac{1}{2}$ minutes after administration of the drug. The records of duodenal contraction during the nausea following the administration of morphine and that following labyrinthine stimulation were quite similar (fig. 3).

As judged from fluoroscopic observation and, more accurately, from the simultaneous recording of both balloons (fig. 4), the contraction which involved the descending duodenum during nausea occurred at the same instant in the proximal and the distal portions. Reverse peristalsis involving first the distal, then the proximal balloon was never observed. Nevertheless, the duodenal contraction expelled both balloons into the stomach in 9 instances without any vomiting taking place. This occurred 6 times after labyrinthine stimulation and 3 times after morphine sulfate was given. As the balloons passed backwards through the pylorus, each contained air in amounts varying between 5 and 15 cc.

Discussion. The rôle of the duodenum during nausea and vomiting has generally been neglected. Hatcher (4) reviewed the mechanism of vomiting in 1924 but failed to mention the duodenum. He apparently felt that pylorospasm initiated the gastro-intestinal reactions during emesis. Best and Taylor (5) emphasize antiperistalsis and "tension upon" the walls of the esophagus, stomach and duodenum. On theoretical grounds, Alvarez (6) has suggested that at times nausea "has its origin in reverse peristalsis in the bowel" but apparently was never able to confirm this hypothesis. Recently Oppenheimer and Mann (7), working on dogs with exteriorized intestinal loops, showed that attacks of emesis provoked by drugs were preceded by a short increase in intestinal activity.

The problem of seasickness has led a few otolaryngologists to investigate the relation between labyrinthine stimulation and intestinal activity. According to Spiegel and Demetriades (8), who studied the surgically exposed intestinal loops of animals, caloric stimulation of the labyrinth increased the extent of small intestinal contractions. LeHeux and deKleyn (9) found that labyrinthine extirpation in the cat enfeebled gastric and, to a lesser degree, intestinal motor activity. These observations, however, have not been applied to the field of gastro-intestinal physiology.

The present experiments show that nausea, whether caused by excitation of the semicircular canals or by the administration of morphine, is frequently accompanied by a generalized contraction of the descending duodenum. The question arises whether some of the symptoms of nausea, such as the desire to vomit, may actually be caused by the duodenal contraction. Though possible, this hypothesis is unlikely since we have shown that a narrowing of the duodenal

lumen does not always attend nausea. Furthermore, as Abbott and Pendergrass (10) have reported, the administration of 0.015 gram of morphine causes small intestinal contraction, but clinically this same dose does not invariably nauseate the patient. Our subjects experienced nausea without fail after receiving morphine, but it must be remembered that they were intubated and had been exposed to labyrinthine stimulation.

Recent experiments by Quigley et al. (11) and by Mecray (12) have corroborated Abbott's (13) contention that reverse peristalsis—i.e., orad progress of a muscular contraction—is rarely seen, particularly in the human small intestine. Our records indicate that during nausea balloons in the descending duodenum are pushed backwards into the stomach even though the "peristaltic" waves continue to travel aborally. A possible explanation of this phenomenon is that the intestinal gradient postulated by Alvarez (14) has been reversed by the generalized but stationary duodenal contraction.

The state of the pylorus is hard to determine by our methods, but obviously complete pylorospasm is not present during nausea or just before emesis. If it were, the duodenum could not expel the partially inflated balloons as easily as it does.

Since the nausea and duodenal contraction produced by labyrinthine stimulation must be of "central" origin, a similar duodenal response may characterize the gastro-intestinal reactions of intra-cranial disorders, of sea or air-sickness, and of migraine. "Bilious spells"—attacks consisting of headache, nausea, and the vomiting of bile-stained material—may also depend upon a "central" mechanism which brings about duodenal spasm and regurgitation of intestinal contents. These "bilious spells," especially when they are accompanied by right upper abdominal distress, are sometimes treated with opiates, but the experiments here presented show why such therapy merely prolongs the symptoms.

SUMMARY

Kymographic records of duodenal activity after labyrinthine excitation and after the administration of morphine sulfate were taken in 20 subjects.

Nausea was produced 13 times by caloric stimulation of the labyrinth and 5 times by morphine sulfate administration. In 13 of these instances, a generalized contraction of the descending duodenum was recorded during nausea.

The contraction of the descending duodenum often expelled both balloons backward into the stomach, although no reverse peristalsis was observed.

It is suggested that duodenal spasm is a frequent concomitant of nausea, and that this spasm pushes the duodenal contents into the stomach by reversing the intestinal gradient. Necessarily, absolute pylorospasm during nausea would then be impossible.

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DIFFERENCES AMONG ADRENAL STEROIDS WITH RESPECT TO THEIR EFFICACY IN PROTECTING THE ADRENALECTOMIZED DOG AGAINST CIRCULATORY FAILURE

W. W. SWINGLE, J. W. REMINGTON,[1] V. A. DRILL AND W. KLEINBERG

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, N. J.

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In previous communications (1, 2) it was pointed out that the synthetic steroid desoxycorticosterone acetate differs qualitatively from adrenal cortical extract with respect to its efficiency in protecting the circulation of the adrenalectomized dog against various shock inducing procedures. For example, stripping the intestine or removal of both adrenal glands at a single stage operation, leads to a slow but steady decline in arterial pressure of both the untreated and desoxycorticosterone treated dog, death occurring within 8 to 24 hours. If, however, the animals are treated with liberal amounts of potent extract, the blood pressure fall is negligible, and normal activity and vigor are maintained. It is evident therefore, that whole extract, unlike desoxycorticosterone, contains factors which afford protection to the circulation of the adrenalectomized dog subjected to these two procedures.

Failure of the circulation which is unresponsive to treatment with desoxycorticosterone is apparently unrelated to alterations in the electrolyte pattern of the blood, renal loss of body fluids, and hemoconcentration. Lowering of the blood sugar is a fairly constant finding following the single stage bilateral adrenalectomy, less so after stripping the intestine. In some cases, however, hypoglycemia may develop. The experiments show that no simple correlation exists between the actual level of blood sugar and the decline in arterial pressure. Intramuscular injections of glucose at frequent intervals and in amounts sufficient to maintain the blood sugar at or near normal, will prolong survival although the blood pressure is not restored. It is probable therefore that marked alterations in carbohydrate metabolism are characteristic responses to these two procedures, the fall in blood sugar merely serving as a rough index of upsets of a more fundamental nature in the metabolism of glucose.

Since desoxycorticosterone is practically without effect upon those phases of carbohydrate metabolism influenced by cortical extract and adrenal steroids having an oxygen at carbon-11, and since it is also ineffective as a prophylactic against types of shock in which blood sugar changes are marked, it was considered essential to repeat earlier experiments (1, 2) using corticosterone and 17-hydroxy-11-dehydrocorticosterone.[2]

[1] Upjohn Research Fellow

[2] We are greatly indebted to Dr. E. C. Kendall, Division of Biochemistry, Mayo Foundation for generous supplies of Compound E.

The corticosterone was obtained from Dr. J. J. Pfiffner, Parke Davis and Co. and Dr. Oscar Wintersteiner, Squibb Institute for Medical Research. Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc. furnished the desoxycorticosterone acetate (Percorten).

The type of animal used and the methods employed for inducing circulatory collapse, and determining blood pressure, have been discussed in earlier papers (3, 4). The corticosterone and 17-hydroxy-11-dehydrocorticosterone (compound E of Kendall's series) were dissolved in 95 per cent alcohol and then diluted with distilled water so that each 1.75 cc. contained 5 mgm. of steroid. This 50 per cent alcohol solution was injected intravenously at a very slow rate. Tests made on both intact and adrenalectomized dogs afforded no evidence that the alcohol *per se* affected the results.

I. *Trauma to the intestine.* The entire length of the small intestine was gently stripped between the fingers for twenty-five minutes using strict asepsis. In the normal dog, this amount of stripping is without demonstrable effect upon the blood pressure or vigor of the animal. The adrenalectomized dog maintained in apparently normal health by cortical extract is extremely sensitive to such treatment, and dies from circulatory collapse within 8 to 19 hours. Fore treatment with desoxycorticosterone is without effect upon the circulation in this type of trauma.

A. *Effect of 17-hydroxy-11-dehydrocorticosterone when used as a prophylactic fore treatment.* So far as the writers are aware no one has tested this steroid in shock produced by trauma to the intestine. Representative data obtained from study of four dogs are given in table 1. The animals at no time exhibited symptoms, remaining active and vigorous from the time of recovery from the anesthetic until the experiment was discontinued. Hemoconcentration occurred in all the animals despite lack of symptoms. The blood sugar levels were elevated above normal. Animals treated with similar doses of desoxycorticosterone usually show a sharp decline in blood sugar and may exhibit hypoglycemic convulsions.

B. *Effect of corticosterone when used as a prophylactic fore treatment.* Selye and co-workers (5) reported that corticosterone will prolong the survival of intact rats shocked by crushing the intestine several times with hemostats. In view of this report, positive responses were anticipated with this steroid so that we were disappointed to find the results quite variable. Of four dogs subjected to intestinal stripping, two responded to the treatment very well, exhibiting no signs of circulatory failure. The blood pressure declined somewhat, but later recovered spontaneously to levels approaching normal (dog 4, table 1). The other two dogs developed circulatory failure and died within 12 hours. These animals reacted as though they had received no treatment whatever. The fact that 50 per cent of the dogs responded to corticosterone whereas 50 per cent did not might be an indication that the method of administration was at fault, i.e., that the corticosterone, unlike 17-hydroxy-11-dehydrocorticosterone, sometimes failed to remain in solution when injected directly into the blood, and was therefore not immediately available to the animal. Whatever the cause for the inconstant results, it must be concluded at present that the action of corticosterone is definitely inferior to that of 17-hydroxy-11-dehydrocorticosterone.

II. *Bilateral adrenalectomy at a single stage operation.* The dog reacts unfavorably to bilateral removal of both adrenal glands at a single stage operation unless

special care is taken to avoid injury to the nervous elements adjacent to the glands (2). The animals develop circulatory failure, rarely surviving for more than 10 to 20 hours. Treatment with desoxycorticosterone does not prolong the life span. If, however, the adrenals and surrounding tissue are thoroughly infiltrated with procaine previous to gland removal, so that afferent impulses arising in the area of injury are locally blocked, circulatory changes do not occur

TABLE 1

Effect of adrenal steroids in protecting against circulatory failure following a twenty-five minute intestinal stripping*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMOGLOBIN	BLOOD SUGAR	REMARKS
		mm. Hg	*per minute*	*grams per cent*	*mgm. per cent*	
Dog 1, 10.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
9/29	12:45 p.m.	106	50	13.1	83	Completed stripping
	5:40 p.m.	105	152	16.7	60	
	11:40 p.m.	102	124	14.6	89	Active
9/30	11:40 a.m.	112	110	15.0	99	Normal, given food and water
Dog 2, 11.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/28	10:30 a.m.	105	100	9.0	85	Completed stripping
	3:30 p.m.	105	168	11.5	98	
	9:30 p.m.	104	180	11.1	107	No symptoms
10/29	10:30 a.m.	104	160	11.5	92	Normal, given food and water
Dog 3, 9.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/21	10:30 a.m.	112	120	14.3	83	Completed stripping. Pressure fell to 62 mm. Hg
	3:30 p.m.	86	196	15.6	60	
	10:30 p.m.	96	168	15.0	96	Strong, given food
11/22	10:30 a.m.	102	148	15.0	88	Appears normal
Dog 4, 11.2 kgm., 20 mgm. corticosterone						
1/10	11:30 a.m.	109	68	12.5	70	Completed stripping
	4:30 p.m.	112	104		71	
	11:30 p.m.	98	188	19.8	66	Strong, active
1/11	9:30 a.m.	93	192	15.3	70	Appears normal

* Given in four intravenous injections of 5 mgm. each at 2 hours before the stripping, and at 2, 5 and 8 hours after the stripping.

and the animals can be easily maintained on desoxycorticosterone. The same thing is true if the spinal cord is sectioned at the level of the first thoracic vertebra previous to adrenal removal.[3]

A. *Efficacy of 17-hydroxy-11-dehydrocorticosterone when used as a prophylactic fore treatment.* Four dogs, given 10 to 20 mgm. in divided doses (table 2), were

[3] Unpublished work.

subjected to bilateral adrenalectomy at a single stage operation. All remained active and vigorous throughout the experimental period of 24 hours, an interval deemed adequate since untreated dogs subjected to this operation always succumb before the 24th hour. The only animal which showed a blood pressure decline was the one that received the least amount of steroid (table 2, dog 7).

TABLE 2

Effect of adrenal steroids in protecting against circulatory failure following single stage bilateral adrenalectomy*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMOGLOBIN	BLOOD SUGAR	REMARKS
		mm. Hg	*per minute*	*grams per cent*	*mgm. per cent*	
Dog 5, 11.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/2	11:00 a.m.	120	100	13.0	89	Completed operation
	4:00 p.m.	128	104	12.4	77	
	11:00 p.m.	114	96	12.7	99	No symptoms
10/3	11:00 a.m.	120	103	13.0	80	Normal
Dog 6, 8.6 kgm., 15 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/6	12:10 p.m.	98	96	11.1	84	Completed operation
	5:00 p.m.	115	126	11.7	79	
	11:00 p.m.	111	108	11.2	100	No symptoms
10/7	11:00 a.m.	109	128	11.1	86	Normal
Dog 7, 10.7 kgm., 10 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/27	4:00 p.m.	122	112	18.5	88	Completed operation
	10:00 p.m.	80	175	17.0	87	Lethargic
10/28	10:00 a.m.	86	180	16.8	85	Inactive. Given cortical extract
10/29	10:00 a.m.	105	120	17.0	86	Appears normal
Dog 8, 9.7 kgm., 20 mgm. corticosterone						
10/29	11:00 a.m.	105	120	13.8	87	Completed operation
	4.00 p.m.	83	184	14.0	90	
	10:00 p.m.	80	240	13.9	96	Inactive
10/30	10:00 a.m.	98	160	13.7	94	Appears normal

* Given intravenously as follows: All dogs received 5 mgm. 2 hours before operation; dogs 5 and 8 received 3 injections of 5 mgm. each at 2, 5 and 8 hours after operation; dog 6 received 5 mgm. at 2 hours and 2.5 mgm. at 5 and 8 hours after operation; dog 7 received 2.5 mgm. at 2 and 5 hours after operation.

Despite the lowered pressure the dog remained active and alert. The response of this animal makes it seem evident that 10 mgm. represents approximately the minimum dose requisite for adequate protection against circulatory failure.

Blood studies revealed no indication of hemoconcentration. The blood sugars were elevated above pre-operative levels.

B. *Efficacy of corticosterone as a prophylactic fore treatment.* Sufficient cor-

ticosterone was available to test only one dog (table 2, dog 8) but this case proved strikingly clear. The arterial pressure fell 25 mm. Hg during the critical part of the experiment (8–12 hrs. following operation) but recovered without further treatment. The animal remained in good condition and ate full rations at the end of 24 hours.

There was no appreciable hemoconcentration as indicated by hemoglobin determinations. The blood sugar was elevated. The protection afforded this single corticosterone-treated dog seemed distinctly inferior to that given by equivalent amounts of 17-hydroxy-11-dehydrocorticosterone.

III. *Trauma to muscle masses.* It was next considered of interest to test the efficiency of 17-hydroxy-11-dehydrocorticosterone in protecting the circulation in those types of shock previously shown to respond well to desoxycorticosterone (1).

Trauma to muscle masses by 100 light blows with a wooden mallet to the hind leg of the deeply anesthetized adrenalectomized dog receiving minimum maintenance doses of cortical extract, results in circulatory failure within 8 to 14 hours. Fore treatment of similar animals with 20 mgm. of desoxycorticosterone prevents any impairment of the circulation.

Owing to the limited supply of 17-hydroxy-11-dehydrocorticosterone available, only two animals were tested (table 3). However, the results were so decisive that more are probably unnecessary to establish the point. Upon recovery from the ether the animals were strong, active and showed no significant changes in arterial pressure. The injured leg rapidly swelled to nearly twice normal size and some hemoconcentration was present. The blood sugar was elevated above normal.

IV. *Ineffectiveness of desoxycorticosterone acetate as a prophylactic for muscle injury when the adrenalectomized dog has been maintained on synthetic steroid instead of natural extract.* It was mentioned earlier that daily maintenance doses of cortical extract (0.3–0.5 cc. per kgm. body weight in which 1 cc. contains the equivalent of 50 grams of fresh cortex) will not prevent the circulatory failure which follows muscle trauma. If the animal is given 10 to 20 mgm. of desoxycorticosterone as fore treatment, 12 to 18 hours before trauma, and extract discontinued, it is able to withstand very severe tissue abuse without symptoms appearing (1). Recently we discovered, quite by accident, that the protective action of desoxycorticosterone is manifest *only in those animals which have been maintained on natural extract for several days previous to injury.* Desoxycorticosterone loses its protective effect upon the circulation when employed as a prophylactic on animals which have been maintained solely on this steroid and hence are completely lacking in the factors concerned with carbohydrate metabolism. Table 3 is a summary of the experimental findings. It will be noted that the blood sugar fell to low levels along with the blood pressure. Definite hypoglycemic symptoms were not observed.

V. *Efficacy of 17-hydroxy-11-dehydrocorticosterone as prophylactic fore treatment for circulatory failure following hemorrhage.* The adrenalectomized dog, receiving maintenance doses of extract and given fore treatment with desoxy-

corticosterone, responds to hemorrhage in all observable respects like the intact dog (2). Large quantities of blood (30–35 cc. per kgm.) can be withdrawn with-

TABLE 3

Effect of adrenal steroids in protecting against circulatory failure following muscle trauma*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMOGLOBIN	BLOOD SUGAR	REMARKS
		mm. Hg	*per minute*	*grams per cent*	*mgm. per cent*	
Dog 9, 9.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/24	9:30 a.m.	115	80	15.0	88	Trauma finished at 10:30 a.m. Pressure fell to 57 mm. Hg
	3:30 p.m.	104	184			
	10:30 p.m.	108	180	16.2	78	Strong, alert, ate food
11/25	10:30 a.m.	122	88	13.5	112	Leg still swollen but dog appears normal
Dog 10, 10.2 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/24	9:30 a.m.	108	88	14.9	88	Trauma finished at 12:30 p.m.
	5:30 p.m.	91	176			
	11:30 p.m.	93	160	15.8	135	Active, ate food
11/25	12:30 p.m.	103	116	11.5	116	Normal except for swollen leg
Dog 11, 9.8 kgm., 10 mgm. desoxycorticosterone acetate, after maintenance with cortical extract						
1/15	10:00 a.m.	111	90	15.5	90	Trauma finished at 10:30 a.m.
	3:30 p.m.	116	148	15.8	92	
	5:30 p.m.	100	168	16.2	97	
	10:30 p.m.	99	176	14.9	96	Good condition, ate food
1/16	10:30 a.m.	116	120	14.0	92	Normal except for swollen leg
Dog 12, 10.0 kgm., 10 mgm. desoxycorticosterone acetate, after maintenance with desoxycorticosterone acetate						
12/12	9:30 a.m.	111	116	10.5	88	Trauma finished at 10:00 a.m.
	2:00 p.m.	48	176	11.0	67	Died at 2:30 p.m.
Dog 13, 8.6 kgm., 20 mgm. desoxycorticosterone acetate, after maintenance with desoxycorticosterone acetate						
1/7	10:15 a.m.	120	108	9.8	83	Completed trauma at 10:30 a.m.
	2:30 p.m.	40	200	10.2	53	Comatose. Died at 3:15 p.m.

* Dogs 10 and 11 given four intravenous injections of 5 mgm. each at 2 hours before trauma, and at 2, 5, and 8 hours after; dogs 12 and 13 given two intramuscular injections at 12 and 2 hours before trauma; dog 14 given four intramuscular injections at 16, 12, 2 and 1 hour before trauma.

out inducing circulatory failure. The animals sustain an intense and prolonged vasoconstriction, the blood pressure remaining above normal throughout 80

per cent of the hemorrhage. During the last 20 per cent of the bleeding the pressure falls rapidly to shock levels. Blood dilution begins immediately and continues until the arterial pressure is restored to normal. The pressure rises spontaneously from shock levels, attaining normal within 24 to 48 hours.

In contrast, the blood pressure of the adrenalectomized dog receiving maintenance extract but no desoxycorticosterone falls steadily from the beginning of the hemorrhage. Removal of only 15 to 17 cc. blood per kgm. body weight suffices to reduce the pressure to shock levels. Upon cessation of the bleeding, the pressure may rise to about 60 mm. Hg, remain at this level for 3 to 6 hours, and then decline fairly rapidly to levels incompatible with life. There is no observable blood dilution during the course of the hemorrhage, although dilution may occur when the pressure reaches the low plateau of about 60 mm. Hg. However, it is never comparable in extent to that seen when liberal amounts of cortical extract or desoxycorticosterone are given previous to bleeding.

The dogs (previously maintained on extract) treated with 17-hydroxy-11-dehydrocorticosterone varied widely as to the total amount of blood which could be removed before the arterial pressure was reduced to 40–50 mm. Hg (table 4), but it totalled less than that which can be taken from the desoxycorticosterone treated animal. In several ways the response of the 17-hydroxy-11-dehydrocorticosterone-treated dogs was similar to non-primed animals receiving only maintenance doses of extract. 1, there was no initial pressure rise above normal; 2, blood dilution did not occur during the hemorrhage; 3, the arterial pressure rose spontaneously from shock levels to about 60 mm. Hg and was maintained at this level for several hours, during which interval blood dilution began. Unlike the untreated dog, however, the pressure then rose slowly but steadily toward normal. One of the three dogs tested (no. 14) failed to show a full pressure recovery by the 48th hour, when the experiment discontinued.

The hemorrhage experiments indicate that 17-hydroxy-11-dehydrocorticosterone is definitely less effective in protecting the adrenalectomized dog against circulatory failure following simple blood loss than is desoxycorticosterone, when the latter is used as a prophylactic on animals previously maintained on small doses of cortical extract. The dogs were not able to withstand the loss of as much blood, and the response to treatment was less dramatic. We have never observed a fall in blood sugar in the hemorrhaged adrenalectomized dog and it is very doubtful if marked alterations in blood sugar levels occur even when the animal is in collapse. Experiments now in progress do indicate, however, that even in hemorrhage the efficacy of desoxycorticosterone in protecting the circulation is reduced to zero when the animal has been maintained solely on this steroid previous to bleeding and hence is completely lacking in carbohydrate-active factors.

Discussion. It seems more than coincidental that the circulatory failures most responsive to treatment with adrenal steroids having an oxygen at carbon-11 (carbohydrate active, 9–11) are at the same time those least affected by desoxycorticosterone (carbohydrate inactive 10–12) and are characterized by a significant decline in blood sugar. This suggests that the circulatory failure is

associated in some way with carbohydrate metabolism. The unexpected finding that desoxycorticosterone loses its protective effect upon the circulation unless the animal has been previously maintained on natural extract (presumably containing steroids with an oxygen at carbon-11) affords support for this general idea. The small daily maintenance doses of extract, although quite inadequate to prevent circulatory failure, apparently do serve to protect the animal against

TABLE 4

Effect of adrenal steroids in protecting against circulatory failure following hemorrhage*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMOGLOBIN	BLOOD SUGAR	REMARKS
Dog 14, 9.7 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone. Removed 17.2 cc. blood per kgm. body weight						
		mm. Hg	*per minute*	*grams per cent*	*mgm. per cent*	
12/1	10:00 a.m.	102	128	13.6	73	
	11:10 a.m.	45	88	13.5		Completed hemorrhage
	2:20 p.m.	67	148	12.0		
	10:20 p.m.	66	148	10.5	94	Still weak
12/2	11:00 a.m.	89	128	9.6	93	Given food, drank water
12/3	11:00 a.m.	79	144	9.0	93	Experiment discontinued
Dog 15, 8.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone. Removed 31.8 cc. blood per kgm. body weight						
12/1	10:00 a.m.	100	132	10.2	85	
	1:05 p.m.	47	84	10.1		Completed hemorrhage
	4:05 p.m.	62	136	10.1		
	10:05 p.m.	62	144	8.8	92	Weak, depressed
12/2	10:05 a.m.	69	116	8.0	95	Given food, water
12/3	10:05 a.m.	94	100	6.7	90	Experiment discontinued
Dog. 16, 8.6 kgm. 20 mgm., 17-hydroxy-11-dehydrocorticosterone. Removed 20.0 cc. blood per kgm. body weight						
12/5	10:00 a.m.	122	72	13.3	83	
	11:30 a.m.	47	98	13.3		Completed hemorrhage
	2:30 p.m.	63	140	13.6	85	
	10:30 p.m.	62	100	12.2	85	Weak, given water
12/6	10:30 a.m.	101	96	11.5	84	Appears normal

* Given in four intravenous injections of 5 mgm. each at 2 hours before the hemorrhage, and at 2, 5 and 8 hours after the hemorrhage.

drastic carbohydrate changes and thereby enable desoxycorticosterone to maintain the circulation.

The carbohydrate upsets involved are seemingly much more subtle than a mere decline in blood sugar level, which, except as to general trend, can not be correlated with blood pressure change. Various workers (7–9) have hinted that the intermediary metabolism of carbohydrate in the tissues themselves is ab-

normal in the adrenalectomized animal. It is tempting, therefore, to ascribe the fundamental disability, in so far as circulatory failure due to stress is concerned, to a breakdown of the carbohydrate cycle in the tissues. This metabolic dysfunction would be manifested by inability of the peripheral vasculature to perform properly, and eventuate in peripheral vascular failure and circulatory collapse.

The carbohydrate active steroids, when present in sufficient amounts, adequately protect the circulation; when present in quantities far too small to afford protection, nevertheless may be requisite for rendering the peripheral vessels responsive to desoxycorticosterone, which lacks the property of influencing carbohydrate metabolism.

The action of small doses of extract, ineffective in themselves, in rendering desoxycorticosterone active might also be accounted for on the assumption of a synergistic action between steroids in natural extract and desoxycorticosterone. Such a synergism can not be the sole explanation for circulatory failure following all stress procedures, however, for local blocking of the nerves by procaine, spinal anesthesia, or section of the spinal cord at the first thoracic vertebra,[3] enables desoxycorticosterone to become an effective agent for protecting the circulation following the single stage bilateral adrenalectomy. Spinal section also prevents the circulatory failure following intestinal stripping.[3] It seems necessary therefore to postulate the participation of a nervous factor in circulatory collapse resulting from these procedures. Nociceptive stimuli arising in the area of injury will, unless prevented by local or spinal anesthesia or spinal cord section, induce peripheral vascular failure, presumably by intense reflex stimulation of the vasomotor centers leading to eventual exhaustion, not of the centers themselves, but of the end organ, i.e., the peripheral vasculature.

The response of the vascular periphery to stimulation by barrages of impulses arising in the excited higher nervous centers would demand an increased metabolism of available carbohydrate. Since both the untreated and desoxycorticosterone-treated adrenalectomized dog lack essential factors for maintaining the normal cycle of carbohydrate metabolism the periphery would soon fail and circulatory collapse ensue.

Spinal section or anesthesia, by effectively blocking afferent impulses to the higher centers and so preventing excitation of these centers, should thereby prevent undue stress on the vascular periphery and, presumably, the depletion of carbohydrate reserves and early fatigue of the peripheral vasculature.

If the above interpretation should be found to be essentially correct, then it would appear that since other tissues of the body of the adrenalectomized dog participate in these disabilities of carbohydrate metabolism, the circulatory failure is but one manifestation of a generalized and fundamental derangement (13).

SUMMARY

1. The effectiveness of corticosterone and 17-hydroxy-11-dehydrocorticosterone in protecting the circulation of the adrenalectomized dog after stress

procedures has been tested, with emphasis on those types of circulatory failure which are not responsive to desoxycorticosterone therapy.

2. Unlike desoxycorticosterone, both corticosterone and 17-hydroxy-11-dehydrocorticosterone are effective after intestinal stripping and after the removal of both adrenal glands at a single stage operation. The action of corticosterone is inferior to that of 17-hydroxy-11-dehydrocorticosterone.

3. 17-hydroxy-11-dehydrocorticosterone is also effective after muscle trauma and hemorrhage, where desoxycorticosterone also shows activity. Its action in hemorrhage is qualitatively different and inferior to that of desoxycorticosterone.

4. Desoxycorticosterone fore treatment is ineffective in preventing circulatory collapse following muscle injury unless the animal has been receiving maintenance doses of cortical extract previous to trauma. The effectiveness of this steroid following hemorrhage is also greatly reduced or entirely lost unless very small amounts of carbohydrate-active material are present.

5. A possible explanation is suggested for the differences in action of adrenal steroids on the circulation based on 1, blood sugar changes which accompany the circulatory failure; 2, the rôle played by such procedures as spinal section, spinal anesthesia, etc., in protecting the circulation; and 3, the ineffectiveness of desoxycorticosterone when carbohydrate-active steroids are completely lacking.

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THE EFFECT OF DEPLETION OF BODY POTASSIUM ON THE TIME OF SURVIVAL AFTER NEPHRECTOMY AND URETERAL LIGATION[1]

STANLEY H. DURLACHER AND DANIEL C. DARROW

From the Departments of Pathology and Pediatrics, Yale University School of Medicine

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Seventy-five years ago, evidence was brought out that potassium is a metabolite which, by accumulation in extracellular fluids, explains part of the toxic effects of complete abolition of renal function (1, 2). Recent work has again emphasized this point (3, 4, 5). The clearest demonstration is brought out by Hoff, Smith and Winkler (6) who showed that the toxic effects are accompanied by electrocardiographic changes which follow the same sequence as poisoning produced by the injection of potassium and each type of electrocardiographic alteration occurs at the same level of serum potassium in the two conditions. Miller and Darrow (7, 8) demonstrated that diets low in potassium made rats more resistant to the toxic effects of potassium. Injections of desoxycorticosterone acetate have a similar effect (9). The mechanism of this protective action is, in both cases, depletion of muscle potassium which enables this cation to enter the muscle when potassium is injected and thus protect extracellular fluids from a lethal rise in concentration.

The present work shows that the period of survival after nephrectomy or ureteral ligation is prolonged in rats subjected to procedures which have depleted body potassium before operation. The mechanism of this prolonged survival is delay in the post-operative rise in serum potassium which is made possible by low concentration of potassium in muscle.

METHODS. Bilateral nephrectomy and bilateral ureteral ligation were used to produce complete abolition of renal function. Ureteral ligation was found to be somewhat unreliable in rats because of the difficulty of being sure of complete obstruction. In the experiments reported, examination at autopsy indicated successful ligation. The nature of individual experiments will be made clear in the presentation of data.

The chemical methods are those applied to similar studies from the pediatric laboratory (7, 8). In the tables serum sodium and chloride are expressed in milliequivalents per liter of ultrafiltrate by use of a Donnan factor of 0.96 and the content of water in serum. The concentrations of potassium are given per liter of serum since data are not available which enable an accurate calculation of the concentration of potassium in the ultrafiltrate. Tissue analyses are calculated per 100 grams of fat-free solids.

The diets used were as follows: 1, Purina dog chow which contains 15 mM K

[1] The work was aided in part by a grant from the Commonwealth Fund. We are also indebted to Ciba Pharmaceutical Products for the desoxycorticosterone acetate used in this project.

per 100 grams, and 2, a synthetic diet containing 1.6 mM K per 100 grams (commercial casein 18, sucrose 25, dextrin 32, Crisco 22, yeast 2, salt mixture 4 parts).

The survival of rats after abolition of renal function. The length of each of the lines in figure 1 represents the period of survival of an individual rat. The experiments are divided into nine categories for which group 1 serves as the

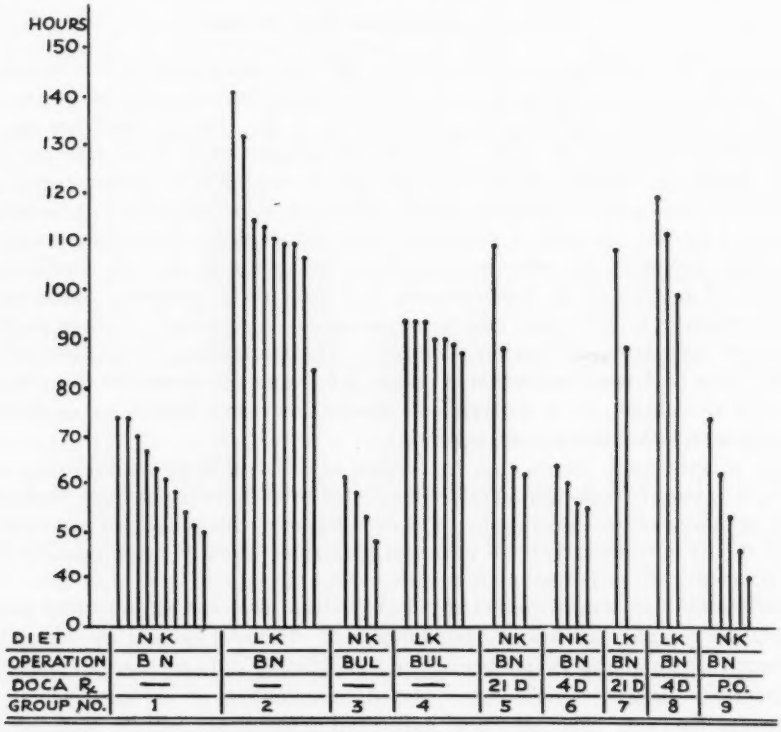

Fig. 1. Survival time after operation

NK = normal potassium diet; LK = low potassium diet; BN = bilateral nephrectomy; BUL = bilateral ureter ligation; 21D = 2 mgm. desoxycorticosterone acetate for 21 days before operation; 4D = 2 mgm. desoxycorticosterone acetate for 4 days before operation; P.O. = 2 mgm. desoxycorticosterone acetate every 24 hours after operation.

control for the nephrectomized rats and group 3 as the control for those subject to bilateral ureteral ligation.

Rats fed a diet of usual potassium content (group 1) survive bilateral nephrectomy for 50 to 74 hours, in this series none living longer than 74 hours. Rats fed a diet low in potassium for 21 days (group 2) survive bilateral nephrectomy for 84 to 141 hours. Since there is no overlapping between groups 1 and 2 the results are perfectly clear cut and indicate that depletion of body potassium by a

diet low in potassium enables rats to survive bilateral nephrectomy longer than rats with normal contents of potassium. These results are not vitiated by intake of potassium, since no food was taken after nephrectomy.

The results are equally clear for the effects of the diet on the period of survival after ureteral ligation (groups 3 and 4). As has been previously pointed out, dogs do not survive bilateral ureteral ligation as long as bilateral nephrectomy (10). The present experiments confirm this observation for rats. The chart shows that rats on a diet low in potassium survive longer than those on a normal diet. This difference is sufficient so that rats fed the diet low in potassium live longer after ureteral ligation than normal rats after nephrectomy. However, the rats on the diet low in potassium survive longer than normal rats when both groups are subjected to ureteral ligation.

The experiments using desoxycorticosterone acetate (groups 5 to 9) show that depletion of body potassium by injections of this compound increases the period of survival and this result is not an effect produced by action of the compound after nephrectomy. Two of four rats of group 5 which ate the normal diet but received 2 mgm. of desoxycorticosterone acetate daily for 21 days previous to operation survived longer than any rat of group 1. Group 3 originally contained six rats but two died at operation probably owing to cardiac lesions produced by prolonged injections of desoxycorticosterone acetate (11). The long survival of the two rats is sufficient to demonstrate the protective action of depletion of body potassium by injection of desoxycorticosterone acetate since it confirms previous observations (12). None of the four rats in group 6 which received 2 mgm. of the compound for 4 days before operation survived longer than 60 hours. Furthermore injection of the compound after operation in group 9 did not prolong the period of survival. The experiments in groups 6 and 9 were intended to bring out any immediate effect of desoxycorticosterone acetate which is independent of the depletion of body potassium. Groups 7 and 8 are supplements to groups 6 and 9 and show that desoxycorticosterone acetate does not alter the results produced by a diet low in potassium. Hence, although group 9 which received injections of desoxycorticosterone acetate after nephrectomy showed a short period of survival in two out of five instances, it is unlikely that this was due to a specific effect of the injection of the compound. Since unpublished data indicate that depletion of body potassium by daily injections of 2 to 4 mgm. of desoxycorticosterone acetate is not great for several days in rats, the contrast between group 5 and 6 shows that the depletion of body potassium rather than an immediate action on tissues is necessary for the protective action of desoxycorticosterone acetate.

Serum and tissue electrolyte after abolition of renal function. Table 1 shows the results of muscle and serum analyses. Certain rats marked with an asterisk were killed when their condition indicated that they would not live longer than one hour. The others were killed at the stated intervals and no exact estimate of the probable period of survival can be given. The table states into which group the various analyses would fit, although only the ones marked with an asterisk were used in estimating the period of survival. From other studies

average figures for serum and muscle are given for 1, normal rats fed a normal diet; 2, normal rats fed a diet low in potassium for three weeks, and 3, normal rats given 2 mgm. of desoxycorticosterone acetate daily for 21 days. In examining the figures from individual rats, changes are noted with reference to one of the three above control analyses of rats which have intact kidneys.

Rats previously fed a normal diet show a fairly rapid rise in the concentration of serum potassium after nephrectomy. The three moribund nephrectomized

TABLE 1

GROUP	NUMBER	DIET	P.O.	SERUM					MUSCLE PER 100 GM. FAT FREE SOLIDS					
				H_2O	NPN	K	[Cl]	[Na]	H_2O	Cl	Na	K	P	N
			hours	*per cent*	*mgm. per cent*	*mM/l.*	*mM/l.*	*mM/l.*	*grams*	*mM*	*mM*	*mM*	*mM*	*grams*
1*	41	NK	N69	93	510	11.9	111	155	354	13.7	13	50	34	14.8
1*	40	NK	N73	94	540	9.9	105	145	393	12.6	16	52	36	15.4
1*	103	NK	N74	94	400	11.5	99	135	343	9.0	10	51	34	14.4
2	31	LK	N42	94		4.7	99	142	345	6.1	14	39	33	15.1
2	29	LK	N43	92		4.9	114	167	313	6.8	15	40	35	15.5
2	30	LK	N90	94	580	5.8	97	156	328	7.9	15	40	33	16.3
2*	27	LK	N113	94	590	8.0	109	157	355	12.6	18	44	33	16.0
2*	28	LK	N114	94	680	5.2	103	151	367	11.8	15	48	35	16.9
3*	42	NK	U48	94		5.3	130	142	342	8.4	11	49	33	15.1
3*	44	NK	U61	93	420	8.1	101	145	376	8.4	11	52	34	16.3
6*	74	NK	N66	94	470	8.7	83	143	381	8.6	13	50	35	16.0
8	66	LK	N76	94	450	4.7	109	152	347	8.8	19	39	34	15.9
8	65	LK	N99	94	515	9.5	100	149	376	11.1	17	45	34	15.8
8*	64	LK	N118	93	505	7.1	105	152	410	16.2	25	39	31	15.9
9	101	NK	N74	94	515	11.4	105	143	393		16	50	33	15.9
9	75	NK	N46	94	396	16.7	101	147	405	12.8	15	51	35	16.6
9*	78	NK	N53	93	424	9.4	125	158	359	8.9	12	50	35	16.4
Control		NK		93	30	4.0	113	147	341	7.2	10	49	33	15.8
Control		LK		93	30	4.0	106	147	333	6.6	15	38	32	15.9
Control D.C.S.A.				93	30	3.7	108	152	328	6.3	15	40	32	15.8

* Moribund.

P.O. hours after nephrectomy (N) or ureteral ligation (U) when rats were anesthetized, bled and killed.

[Cl] and [Na] indicate concentration per liter of ultrafiltrate of serum.

Diet is normal content of potassium (NK) or low in potassium (LK). The latter was given 21 days before operation.

rats, 40, 41 and 103, have values of 10 or more m. eq. per L. which is the concentration accompanied by serious electrocardiographic changes. The muscles of these rats show potassiums that have risen from the normal value of 48 to 50 or more m. eq. per 100 grams of fat free solids. If one assumes that the ratio of muscle chloride to the concentration of chloride in extracellular fluid measures approximately the volume of extracellular water, then normal rats have 2 to 3 m. eq. of non-extracellular sodium (probably intracellular). With the increase

in muscle potassium, evidence of non-extracellular sodium disappears. Of the two rats fed the normal diet and then subjected to ureteral ligation, rat 44 which was killed after 61 hours showed changes like those of the rats subjected to nephrectomy, while rat 42 shows normal serum and muscle potassium because the animal became moribund before the chemical changes had time fully to develop. Thus in normal rats, metabolism frees enough potassium to raise extracellular concentrations to toxic levels within 60 to 75 hours after abolition of renal function.

The rats which received a diet low in potassium (31, 29, 30, 27, 28) present a contrast, for rats 30, 27 and 28 have no or only slight elevation of serum potassium in spite of survival for 90, 113 and 114 hours. Only one of these animals (28) had the usual level of muscle potassium while those of rats 29, 30 and 31 were still abnormally low and that of rat 27, low normal. Muscle chloride is high in the two moribund rats (27 and 28) with normal muscle potassiums. In these animals the muscle sodium gives no evidence of abnormally high intracellular sodium. In the remainder of the rats of group 2, muscle chloride is normal but muscle sodium is high, showing an excess of intracellular sodium.

The nephrectomized rat 74 which had been on the normal diet and had received 2 mgm. of desoxycorticosterone acetate for 4 days shows muscle and serum analyses essentially like uninjected rats. Those receiving desoxycorticosterone acetate after nephrectomy (75, 78 and 101) do not differ from simple nephrectomized rats except perhaps rat 75. This rat became extremely sick within 15 minutes after a subcutaneous injection of the synthetic compound. The explanation of the sudden collapse is the high value for serum potassium (16 mM per L.). Since this result was not a regular occurrence, it does not represent a specific action of desoxycorticosterone acetate.

Discussion. Obviously potassium poisoning is only one of the modes of exit after nephrectomy or ureteral ligation. It is interesting that by delaying the onset of the rise in concentration of potassium in serum, the period of survival is almost doubled. Histological studies show no lesion in the other tissues after nephrectomy. However, after ureteral ligation, foci of necrosis in cardiac and smooth muscle can be demonstrated. Since there is no difference in the various groups in this respect, the rise in concentration of serum potassium does not play an important rôle in the production of these lesions. The animals receiving desoxycorticosterone acetate, particularly if on a diet low in potassium, show extensive areas of necrosis in the heart muscle and replacement by fibrous tissue. These lesions are similar to those seen in rats fed a diet low in potassium (13, 14, 15) or those receiving large doses of desoxycorticosterone acetate (11) and have no relation to abolition of renal function. The rats on a diet low in potassium or receiving the synthetic hormone had large kidneys (19). Thus the diet low in potassium or the injection of desoxycorticosterone acetate produces certain changes in the heart and kidneys but the effect of these procedures does not alter or prevent histological lesions produced by ureteral ligation. The increased survival is apparently entirely connected with the chemical and functional changes accompanying the rise in concentration of potassium in serum.

The mechanism of the protective action of the diets low in potassium and injections of desoxycorticosterone acetate is relatively simple. It is the same as the protective action against injections of KCl which may be produced by depletion of muscle potassium (7, 8). Briefly, our preveious studies have indicated that normal muscle potassium tends to vary between 44 and 49 mM per 100 grams of fat free solids. When it drops below this figure, serum potassium tends to be abnormally low, and when it is above 50, serum potassium is abnormally high. In normal muscle there are apparently 2 to 3 mM of intracellular sodium per 100 grams of fat free solids. As mucle potassium increases, this intracellular sodium disappears; as muscle potassium reaches low figures, intracellular sodium increases. By various means we have been unable to increase muscle potassium beyond 53 mM per 100 grams, since high values were accompanied by concentrations of potassium in serum which produced heart block. The ceiling, however, may actually be related to the amount of intracellular sodium which potassium may displace. At least, rats with high intracellular sodium and low potassium can receive larger injections of KCl before there is a fatal rise in the concentration of potassium in serum. Since the nephrectomized rats do not eat, consumption of body stores frees potassium and the ability of the muscle to absorb potassium is the chief protection against a lethal concentration of potassium in serum. In rats fed a normal diet, the amount of potassium which the muscles can absorb is so small that potassium poisoning is usually the limiting factor in survival after nephrectomy. In rats previously fed a diet low in potassium or those treated preoperatively with desoxycorticosterone acetate, the amount of potassium which may be taken up by the muscle is sufficiently large so that survival is longer and potassium poisoning only occasionally develops.

Selye and Nielson (12) showed that the injection of 10 mgm. of desoxycorticosterone for four days before nephrectomy increased the period of survival. In our experiments 2 mgm. over a similar period was ineffective. There is other evidence that the negative balance of potassium is increased by large doses of the synthetic hormone over that obtained by smaller doses (17, 18). The doses which we used have been shown to reduce muscle potassium to 38 to 42 mM per 100 grams of fat free solids in ten days and about 35 in 21 days.

The serum sodiums and chlorides give some evidence of loss of intracellular sodium from muscles since, in most cases, the concentration of chloride in serum ultrafiltrate decreases while the concentration of sodium remains relatively constant. However, there is only slight evidence that this difference is more marked in the rats fed the diet low in potassium than in the rats on the normal diet.

The rise in non-protein nitrogen is the same in all groups, so depletion of body potassium does not affect this phase of experimental uremia.

Since Hoff, Smith and Winkler (6) pointed out that fatal rise in serum potassium is unlikely in clinical uremia, the present studies have little direct bearing on the problems of nephritis. Specifically diets low in potassium would not be expected to prolong the life of a nephritic patient. However, administration of large doses of potassium salts as a diuretic might be dangerous in certain patients.

SUMMARY

Rats depleted of body potassium by a diet low in potassium or by preoperative injection of desoxycorticosterone acetate survive nephrectomy or ureteral ligation longer than rats on a normal diet. This phenomenon is accompanied by a delay in the rise of serum potassium to toxic levels. This delay in rise of serum potassium is explained by an increase in muscle potassium from abnormally low values towards normal values.

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EFFECTS OF THE REMOVAL OF THE ANTERIOR LOBE OF THE HYPOPHYSIS ON SOME RENAL FUNCTIONS

H. L. WHITE[1], PETER HEINBECKER AND DORIS ROLF

From the Departments of Physiology and Surgery, Washington University School of Medicine, Saint Louis, Missouri

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We have reported (1, 2) that diodrast[2] (D) and inulin plasma clearances and maximum tubular output of diodrast (DT_m) are decreased by hypophysectomy, diodrast and inulin extractions are unchanged. The present paper extends these observations. We are here reporting on 12 dogs; of these 6 had total hypophysectomies (removal or destruction of all of hypophysis, including stalk and median eminence), 4 had simple hypophysectomies (section of stalk with removal of dependent gland), and 2 which showed diabetes insipidus, had been rendered so by appropriate hypopthalamic lesions without any direct operation on the hypophysis. All operations were carried out through the oral approach. In half of the animals, which included representatives of all the classes, the lesions were checked by examination of serially cut sections of the hypothalamus and sella turcica with its contents. The effects of the operative procedures on D and inulin plasma clearances and renal extractions, on renal plasma flow (RPF), on DT_m, on blood volume and on the patency of glomeruli will be considered.

PROCEDURE. Trained female dogs in the postabsorptive state were used. They were on a diet of 1 pound of raw horsemeat daily with Purina dog chow ad lib. Plasma inulin levels of 8 to 14 mgm. per cent were attained by giving 0.34 gram of inulin per kilo as a 25 per cent solution in saline subcutaneously about 90 minutes before beginning the experiments; with hypophysectomized dogs showing lower than normal clearance, about two-thirds of this dose is given. Plasma D iodine levels of 1 to 2 mgm. per cent were attained by giving 0.25 cc. of D solution (35 per cent) per kilo subcutaneously (diluted 1 to 3 with saline) about 45 minutes before an experiment.

Immediately following D administration, 2.5 per cent body weight of water was given by stomach tube. Plasma D levels suitable for DT_m determinations were attained by giving an intravenous priming dose of 0.66 cc. of D per kilo, followed by a sustaining injection of 2 cc. per minute of solution containing 0.5 cc. D solution per kilo per 100 cc. with saline as diluent; with hypophysectomized dogs the sustained dose is 60 per cent of the normal. Sustaining infusion is carried out for 25 minutes before first urine collection is begun; in most clearance experiments 3 or more urine collections were made, 2 or more collections being made in the T_m experiments. The urine collection periods were 20 to 30 minutes; the bladder was washed twice with 30 cc. of saline and urine plus

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[2] The diodrast for this investigation was supplied through the courtesy of the Winthrop Chemical Company.

washings were made up to 250 cc. Mean plasma levels were estimated from curves through 3 or more observed points; with subcutaneous administration fairly constant plasma levels and quite smooth curves are obtained. D was determined by the method of White and Rolf (3). Inulin was determined by a modification of Corcoran and Page's method (4) (the readings are made in photoelectric colorimeter at a volume of 7 cc.). One centimeter of filtrate and 3 cc. of diphenylamine solution (7.5 cc. of 20 per cent diphenylamine in 95 per cent ethyl alcohol plus 56 cc. of ethyl alcohol plus 62.5 cc. of concentrated hydrochloric acid) are put into a boiling water bath for exactly 15 minutes. The

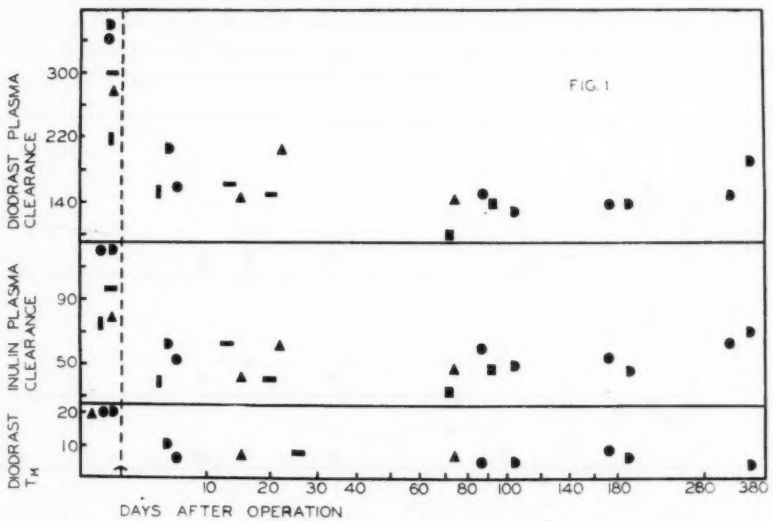

Fig. 1. Plots of DT_m in mgm. per min. per sq. M., inulin and D plasma clearances, cc. per min. per sq. M. before and after operation for completely hypophysectomized dogs. Each point on the clearance plots represents an average of 3 consecutive 20 to 25 minute periods, each point on the T_m's the average of 2 or more consecutive 20 minute periods. The surface areas for all charts were calculated on the basis of the dogs' original weights because it was assumed that no change in kidney's weight would accompany the obesity that developed in the animals because of the nature of their hypothalamic injuries.

tubes are quickly cooled in ice water and made up to 7 cc. with 95 per cent alcohol. Great care is taken in calibration of these tubes. The blue solution is transferred into another test tube for reading, all readings being made in the same test tube by means of a photoelectric colorimeter.

RESULTS. *D plasma clearance.* The results are shown in figures 1, 2 and 3. It is seen that after loss of anterior lobe the clearance falls, being definitely lowered by the 5th to 7th postoperative day on both the complete and simple hypophysectomy dogs (figs. 1 and 2); this fall persists apparently permanently. The values after operation are about half the normal. The average of 45 periods taken on or after the 5th postoperative day with 5 complete hypophysectomy

dogs shows a D plasma clearance value of 155 cc./minutes/M^2, as compared with a normal average of 237 on the same dogs. That the fall is due to loss of the anterior lobe is shown by the findings that *a*, the results are essentially the same after complete hypophysectomy as after the simple, where part of the *pars nervosa* remains, and *b*, there is no fall on destruction of the *pars nervosa* only (fig. 3), except that there may be a transitory rise followed by a transitory fall with a return to a persistent normal.

The fall in D clearance after loss of the anterior lobe begins too early to be ascribed to a fall in metabolic rate known to occur in 6 to 8 weeks in hypophysectomized animals. That there is at first an actual deficit of renal blood flow with respect to the body's needs is further indicated by the finding, based on a limited number of observations, that the plasma urea level rises and is elevated for a few weeks after which it returns toward normal without any increase in D clearance.

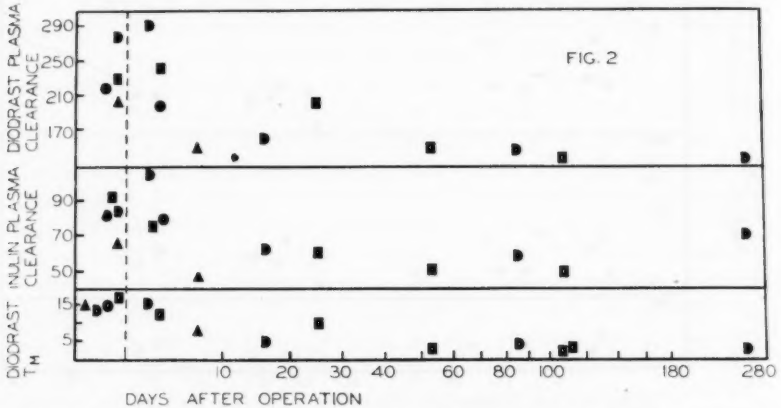

Fig. 2. Plots of DT_m in mgm. per min. per sq. M., inulin and D plasma clearances, cc. per min. per sq. M. before and after operation for simply hypophysectomized dogs. Each point on the clearance plots represents an average of 3 consecutive 20 to 25 minute periods, each point on the T_m's the average of 2 or more consecutive 20 minute periods.

This may mean that as the nitrogen metabolism diminishes several weeks after the operation, a previously inadequate renal blood flow becomes adequate.

Neither simple nor complete hypophysectomy has any effect on the renal extraction of D nor the tubular extraction of D at plasma levels suitable for clearance determinations—White, Heinbecker and Rolf (2). We have no observations on D extractions by diabetes insipidus dogs with intact anterior lobes, but since neither D plasma clearance nor DT_m is changed here and since there is no difference in extractions with simple and complete hypophysectomy, there is no reason to suppose that the *pars nervosa* has any effect on D extractions.

RPF. Since the extraction of D is not affected by hypophysectomy, the expression D plasma clearance/D plasma extraction $\times$ 1.2 (White, Heinbecker and Roff (2), White and Heinbecker (1)) is equally applicable to normal and to hypophysectomized dogs. This is found to give good agreement with the

inulin plasma clearance/inulin plasma extraction. Since it has been established by White and Heinbecker (1) that D plasma clearance × 1.2 shows satisfactory agreement with simultaneously determined RPF, provided extraction is normal, it is possible to estimate RPF on hypophysectomized as well as normal dogs without obtaining renal vein blood.

Inulin clearance. After complete or simple hypophysectomy the inulin clearance falls within a few days and remains permanently low (figs. 1 and 2). The average of 45 periods taken on or after the 5th postoperative day with 5 hypophysectomized dogs shows an inulin plasma clearance value of 54.2 cc./minute/M², as compared with a normal average of 104 on the same dogs. The

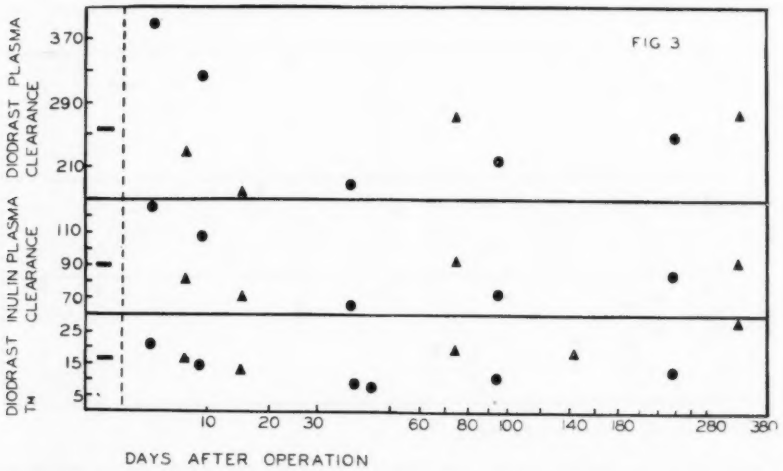

Fig. 3. Plots of DT_m in mgm. per min. per sq. M., inulin and D clearances, cc. per min. per sq. M. before and after operation for dogs with the anterior lobe in, the pitressin forming tissue functionless. Each point on the clearance plots represents the average of 3 consecutive 20 to 25 minute periods, each point on the T_m plot represents the average of 2 or more 20 minute periods. The points for the normals represent the average of all values for our experimental dogs.

average of 21 periods taken on or after the 7th postoperative day with 3 simple hypophysectomy dogs shows an inulin plasma clearance of 54 cc./minute/M², with a normal average of 80 on the same dogs. The filtration fraction is not significantly changed by either type of hypophysectomy. We do not believe that there is a significant difference between the effects of simple and complete hypophysectomy on the D and inulin clearances.

The dogs in which diabetes insipidus was produced by hypothalamic puncture without operative injury to the anterior lobe show a slight fall in unulin clearance, with a return to normal; there may be an early rise preceding the fall. In general, the present findings agree with our earlier report (5) on creatinine clearances in diabetes insipidus dogs.

Diodrast T_m. After complete or simple hypophysectomy DT_m falls rapidly; within a few days it is about half the normal, with a subsequent further fall (figs. 1 and 2). The average of 26 periods on or after the 5th postoperative day with 4 complete hypophysectomy dogs gives a DT_m value of 5.5 mgm./minute/M^2, where the normal had been 21 on the same dogs. The average of 16 periods on or after the 7th postoperative day with 3 simple hypophysectomy dogs shows a DT_m figure of 4.5 mgm./minute/M^2, where the normal had been 15.4 on these dogs. There is no tendency to return toward normal. There is no permanent effect on DT_m in the diabetes insipidus dogs with intact anterior lobes (fig. 3). There may be some initial fall but there is a return to normal within a few weeks.

Blood volume. The effects on blood volume of the loss of the glandular hypophysis, of all pitressin forming tissue and of both together were determined by

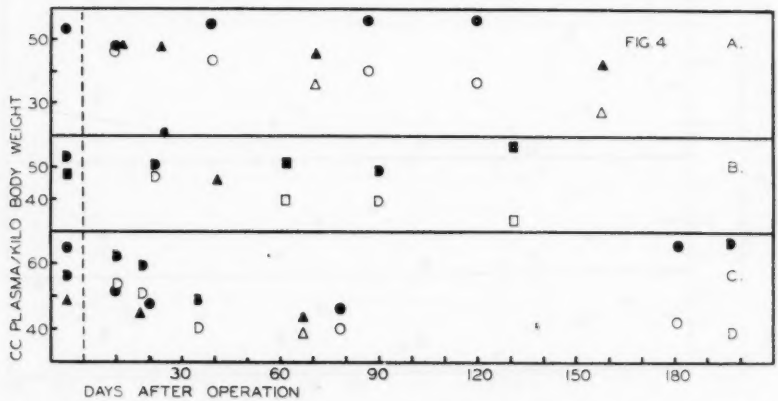

Fig. 4. Plots of plasma volume per kilogram body weight of (A) 2 dogs with intact anterior lobes, pitressin forming tissue functionless, (B) 2 simply hypophysectomized dogs, (C) 3 completely hypophysectomized dogs. Solid marks represent plasma volumes calculated on the basis for stabilized original weights, open marks represent plasma volumes calculated on the basis for actual weights at time of observations.

the blue azo dye (T-1824) method as described by Gibson and Evelyn (6). The values are given as cubic centimeters of plasma per kilo body weight. Because many of the animals gained weight during the period of observation the plasma volumes were calculated on the basis of the original stabilized normal weights and on the actual weights of the animals at the time after operation at which the observations were made.

Analysis of the results (fig. 4) indicates that the blood volume calculated on the basis of the original weights of the animals is unchanged or slightly increased by loss of the glandular or neural hypophysis. Calculated on the basis of the actual body weights of the animals at the time of the observations there is a diminution of blood volume, the degree of which in general varies from 20 to 80 per cent of the percentage increase in body weight.

The amount of decrease in blood volume calculated on the basis of the actual weight at the time of the observation is not more than 5 to 10 per cent until 6 to

8 weeks have elapsed since the removal of the anterior lobe. The depression of D and inulin clearances and particularly of DT_m is fully developed and stabilized at a low level well before a decrease in blood volume has developed. It follows that the decrease in renal function following anterior lobe loss is not to be attributed to a decrease in blood volume.

Glomerular intermittence. In 2 totally and in 2 simply hypophysectomized dogs the phenomenon of glomerular intermittence was looked for according to the method outlined by White (7). Examination of many sections, two of which are shown in figure 5, failed to reveal evidence of any glomerular intermittence. The diminished renal function exhibited by animals deprived of their glandular hypophysis apparently cannot be attributed to glomerular intermittence.

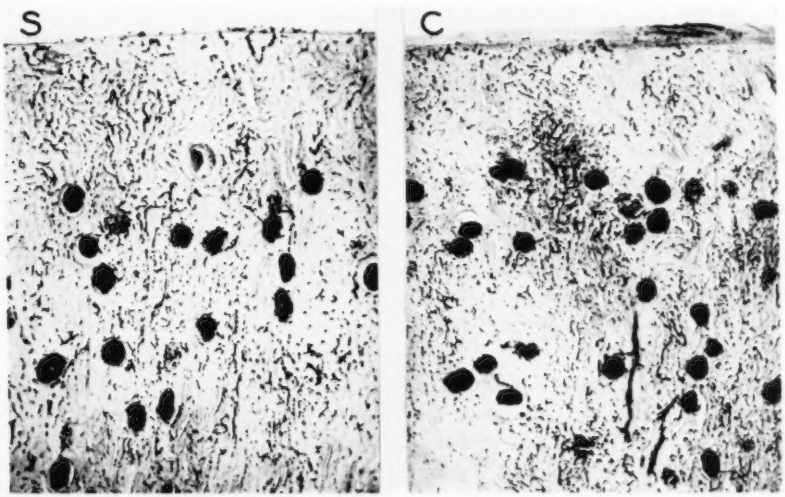

Fig. 5. Photomicrographs 45 diameters showing kidney glomeruli in dogs after ink injection. S—after simple hypophysectomy. C—after complete hypophysectomy. Note all glomeruli are well injected.

Blood pressure determinations. Blood pressure readings made by the auscultatory method on 4 simple and 4 total hypophysectomy dogs have shown that there is no increase and frequently a 10 to 15 per cent decrease in systolic and diastolic pressures four to six months after operations. This is of interest in view of the 50 per cent diminution in renal blood flow shown to be present in these two classes of animals.

Discussion. The results demonstrate that the anterior lobe of the hypophysis plays an important rôle in the maintenance of normal renal blood flow. Its loss results in an average decrease in blood flow of approximately 50 per cent in 4 to 6 days and this reduction has been maintained throughout the period of our observations of 9 to 12 months. The quick onset of the change proves that the result is not secondary to thyroid or adrenal cortical atrophy.

In our experiments exact determinations have been made only of renal blood

flow. Roentgenograms of the hearts of four dogs showing diabetes insipidus, two with, and the others without, the adeno hypophysis show definitely in the latter evidence of a decrease in amplitude of the cardiac contractions and in cardiac output and hence in general blood flow. It is doubted that this method in dogs is sufficiently quantitative for accurate measurements. However, some decrease in general blood flow may be assumed because of the decrease in basal metabolic rate known to follow removal of the adeno hypophysis. This decrease has been shown to be about 16 per cent in dogs by Houssay et al. (8). It comes on gradually in 6 to 8 weeks. Support of evidence of a decrease in general blood flow by Aschner (9) in showing that in hypophysectomized dogs subcutaneously administered epinephrine has little effect on blood sugar whereas when administered intravenously, Russel and Cori (10) or intraperitoneally, Heinbecker and Weichselbaum (11), it has a normal influence. On the other hand the decrease in renal blood flow is established at nearly its maximum level of 50 per cent within a week. It appears then that the depression in renal blood flow is essentially an expression of a loss of a specific influence on the kidney by the adeno hypophysis. Also it has been observed by us that in the dog deprived of *pars distalis*, the quantity of intravenously administered nembutal required to produce comparable anesthesia is approximately half that required by the normal.

The decrease in renal functions evidenced through depression of the inulin and diodrast plasma clearances is attributed to the diminished renal blood flow. The relatively greater decrease in the DT_m cannot be attributed solely to the decrease in blood flow but must reflect an actual decrease in the capacity of the tubules to transport diodrast at high plasma levels.

The interesting observation that in 2 or 3 days following manipulation of the anterior lobe of the hypophysis there may occur an actual increase in inulin and D clearances and in DT_m suggest that extracts of the anterior lobe may possibly be used to stimulate renal functions. This subject is at present under investigation.

SUMMARY

Renal function studies on 12 dogs are reported. Six of the dogs had a total hypophysectomy, 4 a simple hypophysectomy and 2 denervation of the entire neurohypophysis with preservation of the anterior lobe.

D and inulin plasma clearances and DT_m were determined in all dogs before and at intervals after operation for 9 months to a year. In some of the animals D and inulin extractions were determined on explanted kidneys.

D and inulin plasma clearances and DT_m are markedly decreased by loss of the anterior lobe of the hypophysis. D and inulin extractions at low plasma levels are unaffected. Loss of the pitressin forming tissue does not affect these renal functions.

The decrease in D and inulin plasma clearances is attributed to a decrease in renal plasma flow. The decrease in DT_m is due not only to the decrease in renal blood flow but also to a depression of the functional capacity of the tubules to transport D at high plasma levels.

Loss of the anterior lobe of the hypophysis or loss of the entire hypophysis does not result in a diminution of blood volume when the calculations are made on the basis of the original weights of the animals. If the dogs become obese because of the nature of the operation to which they were subjected the blood volume calculated on the basis of actual weight at the time of the observation shows a decrease averaging 50 per cent of the percentage increase in body weight.

Loss of the anterior lobe of the hypophysis does not result in glomerular intermittence.

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RELATION OF THE ADENO AND THE NEUROHYPOPHYSIS TO INSULIN SENSITIVITY AND SUGAR TOLERANCE IN THE DOG

PETER HEINBECKER, H. L. WHITE[1] AND DORIS ROLF

From the Departments of Surgery and Physiology, Washington University School of Medicine, St. Louis, Missouri

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Geiling, Campbell and Ishikawa (1) reported that posterior lobe extracts protected hypophysectomized dogs against the hypoglycemic action of insulin. They reported also that removal of the anterior lobe did not render the dogs insulin sensitive. Houssay and Potik (2) attributed insulin sensitivity in the toad to loss of the anterior lobe and they showed that transplants of anterior lobe protected against insulin hypersensitivity. Pencharz, Cori and Russel (3) reported that removal of the posterior lobe of the hypophysis with only slight injury to the anterior lobe did not increase the sensitivity of rats to the convulsive action of insulin. Removal of the anterior lobe only, leaving the posterior lobe intact, resulted in a marked increase in insulin sensitivity, equal to that observed after complete hypophysectomy. The conclusions of Houssay and Potik in the toad and of Pencharz, Cori and Russel in the rat generally are assumed to apply throughout the animal kingdom in spite of the contradictory evidence of Geiling, Campbell and Ishikawa for the dog.

Operative procedures involving the hypophysis of a type permitting the functional study of its glandular and neural divisions separately were carried out on dogs to investigate their insulin sensitivity and glucose tolerance to orally and intravenously administered glucose. The operated dogs fell into three classes: 1, those "simply" hypophysectomized, i.e., removal of the anterior and posterior lobes; 2, those completely hypophysectomized, i.e., removal of the entire adeno and neurohypophysis (median eminence included); and 3, puncture dogs, those in which by appropriate puncture the entire neurohypophysis was rendered functionless with minimal injury to the anterior lobe. The inference that the anterior lobe functions normally is based on the demonstration of a normal renal blood flow. It has been shown that in such animals loss of the anterior lobe decreases renal blood flow in the dog by 50 per cent (White and Heinbecker, 4).

Two animals of classes 2 and 3, one simple hypophysectomy and one normal dog are reported on. The operations on these animals had been carried out 9 to 12 months previously. Many other simple hypophysectomy and normal dogs have been previously reported on with results similar to those herein given (Weichselbaum, Heinbecker and Somogyi, 5; Heinbecker, Somogyi and Weichselbaum, 6). The insulin and glucose were administered in the fasting state; the amounts together with the route of administration are indicated in the legends of the figures. True sugar values were determined according to the Shaffer-Somogyi method. The animals had been kept on a diet of Purina chow and

[1] Recipient of a grant in-aid-of-research by the Commonwealth Fund.

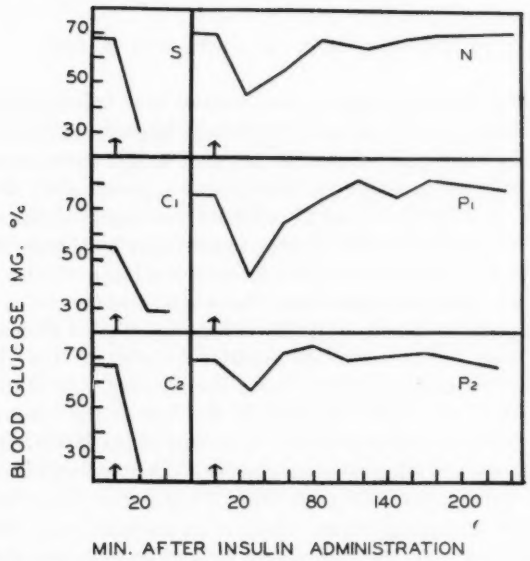

Fig. 1. Insulin response in normal, *N*, simple hypophysectomy, *S*, complete hypophysectomy, *C*, and puncture *P* dogs; 0.125 unit insulin per kilo given intravenously. In all animals without the adenohypophysis hypoglycemic manifestations developed necessitating discontinuance of the experiment and the administration of glucose.

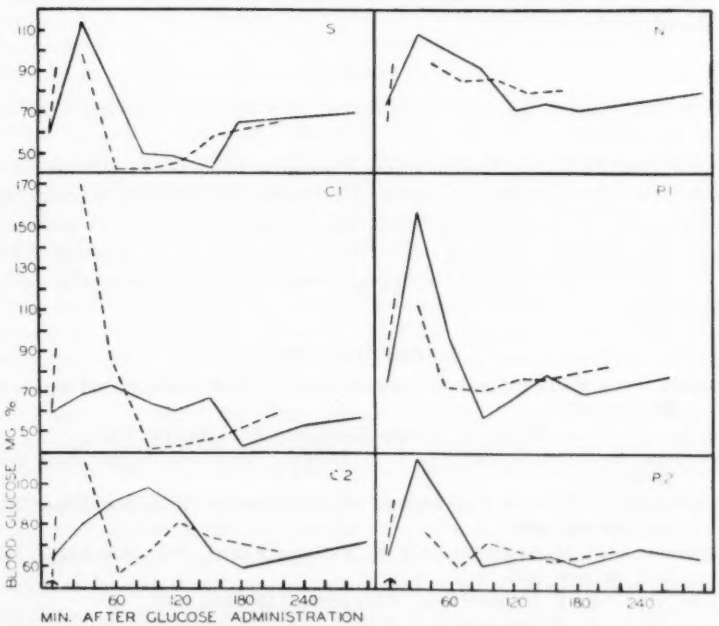

Fig. 2. Glucose tolerance curves in normal, simple hypophysectomy, complete hypophysectomy and puncture dogs after the oral and intravenous administration of glucose. Oral dose 0.85 gram per kilo, intravenous dose 1.5 grams per kilo, —— after oral, ----- after intravenous, administration.

horse meat. The dosages of insulin and glucose were calculated on the basis of the original stabilized weight because the totally hypophysectomized and "puncture" dogs had become quite obese (80 per cent weight increase on an average), the simply hypophysectomized dogs had gained approximately 20 per cent.

Analysis of our results (figs. 1 and 2) indicates that dogs with the adenohypophysis removed tend to show a lower fasting blood sugar level than the normal.

Insulin sensitivity is shown to be due entirely to a loss of the adenohypophysis. The degree of this sensitivity is so great that even after the use of a small testing dose, 0.125 unit per kilo, the experiments on dogs deprived of their adenohypophysis had to be terminated within 30 minutes because of the development of salivation, rapid respiration, stupor and a loss of ability to stand.

No definite effect on sugar tolerance of the loss of the adenohypophysis or neurohypophysis alone is demonstrable by means of the blood sugar response to orally or intravenously administered glucose. Dogs deprived of one or both divisions of the hypophysis show a measure of instability of the blood sugar level in that the degree of hypoglycemia which even normally may follow hyperglycemia is exaggerated. In the completely hypophysectomized dogs the glucose curves following oral administration tend to be flat. This might reasonably be attributed to a slowness of absorption since a diminution in blood flow has been shown to exist in such animals (White, Heinbecker and Rolf, 7). However, this flatness is not shown in the simply hypophysectomized animals which have a similarly decreased blood flow.

SUMMARY

The fasting blood sugar of simple and of total hypophysectomy dogs is lower than in the normal.

Insulin sensitivity in such dogs is due entirely to a loss of the adenohypophysis.

No definite effect on sugar tolerance of the loss of the adeno or neurohypophysis is demonstrable by means of the blood sugar response to orally or intravenously administered glucose. Loss of one or both divisions of the hypophysis results in an exaggeration of the degree of hypoglycemia which even normally may follow hyperglycemia.

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PRODUCTION OF INSULIN RESISTANCE IN DEPANCREATIZED DOGS

JAMES A. GREENE, ANN DAVID AND GEORGE JOHNSTON

From the Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City

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Infection is the most common cause for insulin resistance in diabetes mellitus, but the mechanism is not understood. Of the several theories advanced to explain this resistance, that of decreased insulin secretion by the pancreas appears to have been more generally accepted. Soskin and co-workers (1) were able to produce insulin resistance in depancreatized dogs, but their animals died during or immediately after the acute experiment. Their results have been criticized because they may have been due to the so-called terminal affair. The purpose of this investigation is to ascertain whether or not insulin resistance can be produced in depancreatized dogs in chronic experiments.

METHOD. Depancreatized dogs were placed in metabolism cages and fed a mixture of ground lean beef muscle, cane sugar, dried pancreas and bone meal. The 24 hour urine excretion was collected and analysed quantitatively for dextrose. The animals were catheterized at the end of each 24 hour period to insure accurate collections. Insulin was administered just before the two daily feedings and in amounts sufficient to make the animals utilize most of the ingested sugar. After the diabetes became stabilized foreign proteins, cultures of bacteria or locally irritating substances were injected subcutaneously, intramuscularly, or intravenously. Typhoid vaccine and typhoid H antigen were administered in doses of from 5 million to 1 billion organisms in single or in daily doses. Skimmed milk was administered in doses of from 5 to 20 cc. in single or in daily injections. Cultures of Bacillus coli were administered intravenously in doses of from 1 to 5 cc. in single injections. Turpentine was injected subcutaneously in single doses of 1 cc. Blood sugar estimations were obtained occasionally in each animal. The nitrogen excretion was studied during several observations. Most of the animals were used for several periods of observation and these periods varied from 12 to 66 days. There were 23 periods of observation on 7 dogs. Two of the dogs were sacrificed at the height of the insulin resistance and complete post-mortem studies were made. Particular attention was directed to the liver and all endocrine glands. All animals were sacrificed at the end of the studies and careful search was made for any pancreatic tissue. Small pieces of pancreas were left at the time of the pancreatectomy in two animals and these were found during the post-mortem examination.

RESULTS. Insulin resistance was produced in varying degrees in 16 of the 23 observations. The number of observations on each animal, the number showing no insulin resistance and the number showing different degrees of insulin resistance are shown in table 1. It is to be noted that only one animal (dog 1)

failed to show any insulin resistance, whereas three of them (dogs 4, 5 and 6) showed some resistance during each period of observation. One animal (dog 4) developed spontaneously an extreme insulin resistance which lasted for 30 days. Later a moderate resistance developed following administration of a foreign protein. The potential dextrose intake and the insulin dosage were kept constant in most instances and the degree of insulin resistance was ascertained by the increase in urinary dextrose. An increase of 10 grams or less of urinary dextrose daily was classified as no insulin resistance, an increase of from 10 to 35 grams was considered to be slight, an increase of 35 to 65 grams daily was classified as moderate resistance and an increase of 65 grams or more was considered to be extreme insulin resistance. The estimated blood sugars increased during the periods of insulin resistance and declined as the resistance subsided. The data obtained during the production of moderate insulin resistance in one animal is shown in table 2. It is to be noted that the urinary dextrose increased from

TABLE 1

DOG	EXPERIMENT NO.	INSULIN RESISTANCE			
		None no.	Slight no.	Moderate no.	Extreme no.
1	3	3	—	—	—
2	4	2	1	1	—
3	2	1	—	—	1
4	1	—	—	1	—
5	2	—	2	—	—
6	5	—	2	3	—
7	6	1	—	5	—
Total.....	23	7	5	10	1

Table shows the number of experiments on each dog, the number showing no insulin resistance and the number showing different degrees of insulin resistance.

an average of 9 grams daily to 71.5 grams daily. There was an accompanying increase in urinary volume and not shown in the table an increased urinary nitrogen. An illustration of extreme insulin resistance is shown in table 3. The data presented from the 3rd of March to the 16th day of May are averages for 3 to 7 day periods. After the insulin dosage reached 80 units daily the injections were made 4 times daily instead of the usual 2 times. The animal was sacrificed on the 17th of May and histological studies of the liver, pituitary, thyroid, and adrenals revealed no significant changes.

An analysis of the effectiveness of the different agents employed in production of insulin resistance is of interest. It will be noted in table 4 that typhoid vaccine does not appear to be as effective as typhoid H antigen. This may be due in part to the fact that the former was employed early in the studies and smaller doses were administered. It is our opinion, however, that the latter is the more efficacious. In addition there is the factor that a tolerance appears to develop to any one foreign protein. As the animal was used for later studies it was noted

that larger and larger amounts of the same substance were necessary to produce an equivalent effect. If, on the other hand, one administered a substance not previously employed such larger amounts were not required. The duration of the insulin resistance also varied from observation to observation. In some instances it was present for only a few days, whereas, in one instance it persisted for 66 days. The duration of the insulin resistance was not related to the amount nor to the method of administration of the foreign protein. The daily administration for several days, however, almost uniformly resulted in the production of insulin resistance. Insulin resistance developed following the subcutaneous injection of turpentine in both instances. It appeared the day of injection,

TABLE 2

DATE	DAILY URINE VOLUME	DAILY URINE DEXTROSE	DAILY POTENTIAL DEXTROSE DIET	DAILY INSULIN UNITS	
	cc.	*grams*	*grams*		
6/9/39	415	7.0	145	24	
6/10	405	6.0	145	24	
6/11	340	7.0	145	24	
6/12	440	12.0	145	24	
6/13	495	11.0	145	24	
6/14	480	14.0	145	24	
6/15	490	20.0	145	24	100 mil. H. antigen i.v. 100 mil. H. antigen i.m.
6/16	525	21.0	145	24	
6/17	470	19.0	145	24	
6/18	500	25.0	145	24	200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/19	650	37.0	145	24	200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/20	775	59.5	145	24	200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/21	930	71.5	145	24	200 mil. H. antigen i.v. 200 mil. H. antigen i.m.

Table shows the moderate insulin resistance in dog 6 following administration of 100 million H. antigen intravenously and intramuscularly, and 200 million H. antigen intravenously and intramuscularly daily.

gradually increased until the third day, subsided rapidly for two days and then increased until it reached its maximum two days later at the height of the local reaction. It then decreased as the local irritation subsided. The response to the administration of living Bacillus coli culture was very similar to that obtained with typhoid vaccine.

The responses obtained in the two dogs in which small pieces of pancreas were permitted to remain at the original operation were no different from those in which pancreatic tissue was not found at post-mortem at the end of the studies.

One animal was permitted to develop a fatty liver as described by Best and

co-workers (2) and attributed to lipocaic deficiency by Dragstedt (3). Insulin resistance did not develop following administration of larger doses of a foreign protein that had previously produced insulin resistance in this animal. Also

TABLE 3

DATE	DAILY URINE VOLUME	DAILY URINE DEXTROSE	DAILY POTENTIAL DEXTROSE DIET	DAILY INSULIN UNITS	
	cc.	*grams*	*grams*		
3/6/37	350	3.5	94	10	
3/7	420	1.7	94	10	
3/8	330	1.0	94	10	
3/9	250	1.0	94	10	
3/10	300	1.0	94	10	40 million typhoid i.v. 40 million typhoid i.m.
3/13	440	0.5	94	5	
3/17	860	61.0	94	8	
3/24	1120	82.0	94	10	
3/31	1400	83.0	94	50	
4/6	680	31.0	94	80	
4/13	925	19.0	94	70	
4/20	1200	79.0	94	80	
4/27	1430	89.0	94	80	
5/4	2000	125.0	119	160	
5/11	1260	89.0	94	200	
5/16	700	11.0	94	280	

Table shows extreme insulin resistance in dog 3 following one administration of typhoid vaccine.

TABLE 4

MATERIAL	NO. OF EXPERIMENTS	INSULIN RESISTANCE			
		None no.	Slight no.	Moderate no.	Extreme no.
Typhoid vaccine	9	5	3	0	1
Typhoid H. antigen	8	1	1	6	0
Skimmed milk	3	1	1	1	0
B. coli culture	1	0	0	1	0
Turpentine	2	0	0	2	0
Total	23	7	5	10	1

Table shows the effectiveness of the different materials to produce insulin resistance.

one dog developed just as much insulin resistance after its liver had been filled with 200 grams of acacia as it did before.

Discussion. These observations demonstrate that insulin resistance can be produced in chronic experiments in depancreatized dogs. They make untenable the theory that there is a decrease of endogenous insulin secretion in such cases. There appears to be a difference in susceptability to production of insulin re-

sistance in the different dogs. It does not develop in all dogs under comparable conditions. It may occur in one dog with the slightest provocation and may be very difficult to produce in another dog. In this respect the diabetes mellitus in depancreatized dogs appears to be similar to that in man. According to Greene and Keohen (4) insulin resistance does not occur in all cases of diabetes mellitus in man during an infection nor following the injection of typhoid vaccine.

The cause for the insulin resistance is not clear. The possibility that aberrant pancreatic tissue was present in our animals and that the insulin secretion by this tissue was decreased during insulin resistance appears extremely unlikely. There was no difference in the response of two dogs in which pancreatic tissue was permitted to remain and in those in which no pancreatic tissue was found at autopsy. In addition it is doubtful that cessation of insulin secretion by small portions of pancreas could account for the extreme alterations of dextrose utilization observed in some of our experiments.

The theory that the exogenous insulin is less effective during insulin resistance is undoubtedly true, but this does not explain the mechanism of insulin resistance. There are several possible explanations all of which remain unanswered. The animal is either unable to oxidize as much dextrose as previously or to store glycogen in the liver or tissues to the previous extent. A fatty liver is known to prevent the storage of glycogen by the liver, but the presence of a fatty liver did not augment insulin resistance in one of our animals. Likewise filling one animal's liver with an extreme amount of acacia did not increase the amount of insulin resistance producible in that animal.

SUMMARY

Insulin resistance has been produced in chronic experiments in depancreatized dogs. The susceptability to insulin resistance varies from dog to dog and in the same dog from one observation to another. Small pieces of remaining pancreas, the presence of fatty liver and filling the liver with acacia are not factors in insulin resistance.

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THE RESPIRATION OF THE DEVELOPING BRAIN

D. B. TYLER[1] AND A. VAN HARREVELD

From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

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This report deals with the oxygen consumption of different parts of the brains of rats during extrauterine development and the glucose utilization of whole brains at various ages.

METHODS. The brain, without the olfactory bulbs, was divided as follows: The pallium and cerebellum were separated from the neuraxis. The latter was cut in two in front of the pons. The caudal part to the first spinal nerve is called the medulla; the rostral part, which includes the corpora striata, is designated as the stem.

Warburg respirometers were used for the measurement of the oxygen consumption. Each of the above parts of the brain was chopped and suspended in tared vessels containing Ringer-glucose solution buffered to pH 7.4 with phosphate (1). The wet weights were determined before placing the vessels in the bath. Care was taken to put approximately the same weight of tissue in all the vessels. Thus with the brains of older rats it was necessary to distribute the pallium and the stem in two or three vessels, while to obtain sufficient tissue in newborn rats it was necessary to pool the parts of the brains of several litter mates. The temperature of the bath was maintained at 38°C. Readings were taken beginning 50 min. after death of the animal. The results are expressed in cubic millimeters of oxygen per 100 mgm. of wet brain per hour.

Glucose utilization was determined on whole chopped brain suspended in Ringer-glucose-phosphate medium (10 cc. of medium for each 200 mgm. of tissue) and shaken in stoppered Erlenmeyer flasks for two hours. Shaffer-Hartman-Somogyi's method was used for glucose determination. The results are expressed as milligram of glucose per 100 mgm. of wet weight per hour.

RESULTS. Figure 1a shows the oxygen uptake of the various parts of the brain during growth. In figure 1b is plotted the oxygen uptake of the entire brain during that same period. These latter values were calculated from the oxygen uptake and the weights of the individual parts of the brain. Because of the large relative weight of the pallium its influence on the metabolism of the whole brain is so great that the curve in figure 1b resembles the curve found for the pallium in figure 1a; the influence of the other brain parts is of course in proportion to their relative weights (table 1).

As has been found by others (2), the oxygen uptake of the whole brain is at its lowest level during the first week of life. A slight fall in respiration occurs between the second and fourth day, which is the result of a similar drop that occurs in the pallium and, to a lesser extent, in the stem (fig. 1a). During the

[1] Hixon Fund Fellow.

first two weeks the medulla has the greatest oxygen uptake and the pallium the lowest. No consistent values could be obtained for the cerebellum for the first week because of its minute size, but there are indications that its respiration is of the same magnitude as that found for the medulla. At the end of the first week a sharp rise in the respiration of the pallium and stem occurs, which continues until a maximum is reached at the 7th and 5th week respectively. There occurs during this period a re-arrangement in the order of the rates at which the different parts of the brain take up oxygen. By the fifth week the stem has the highest respiration, followed by the pallium, cerebellum and the

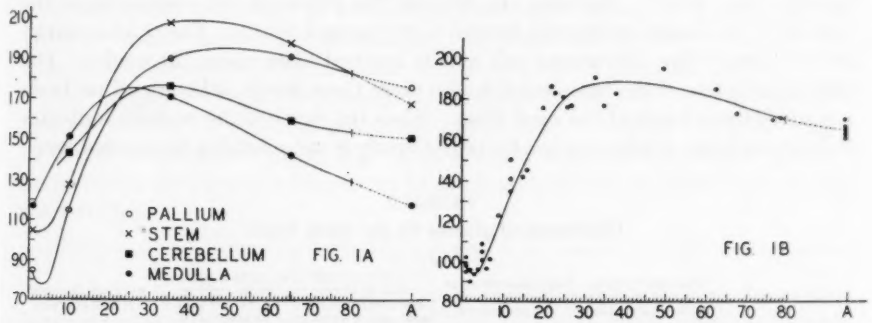

Fig. 1. Oxygen is expressed in cubic millimeters per 100 mgm. of wet brain tissue per hour. Time is given in days. *A* stands for adult.

In figure 1a the points are identification points for the curves of the various parts; they do not represent points of observation. In figure 1b each point is an observation.

TABLE 1

Weight of various parts of the brain (whole brain is 100)

AGE	PALLIUM	STEM	MEDULLA	CEREBELLUM
1–7 days	58	25.5	12.5	4
21–28 days	61	17	11	11
Adult	55	19	14	12

medulla. The medulla showing a steady rise from birth reaches a maximum at about the third week, which is earlier than that of stem or pallium.

The greatest oxygen uptake of the whole brain is found between the 4th and 7th week and then falls off gradually until at about the 20th week the adult level is reached. The respiration of the stem drops below that of the pallium at about the 11th week, so that when the adult level is reached the brain parts take up oxygen in the following order: pallium stem, cerebellum and medulla. This confirms results on adult rats by Himwich, Sykowski and Fazekas (3).

The glucose utilization of three age groups is shown in table 2. It is lowest in the 1 to 10 day old rats, highest in the 10 to 30 day old groups and falls off slightly for the adults. Expressing the glucose and oxygen data in micro-

equivalents (columns IV and V) we find, however, that the ratio of glucose disappearing over the oxygen utilized is greatest in the 1 to 10 day old group (column VI). This glucose oxygen ratio is undoubtedly an index of the amount of glycolysis.

Discussion. The curves of the oxygen consumption of the various parts of the developing rat brain resemble curves reported for dogs (4). In rats there can be distinguished three levels of metabolism during the postnatal life. An examination of the results of Himwich and Fazekas reveals that the same three levels can be found also in dogs. The metabolism during the first week of life is characterized by a very low oxygen and glucose utilization and a high glycolytic activity (first level). Between the 4th and the 7th week the respiration is the highest to be found during the animal's life (second level). The third or adult level is lower than the second one and is reached after about 20 weeks. The individual parts of the brain exhibit also these three levels although all parts do not reach these levels at the same time. Thus the curve of the medulla indicates that at birth the respiration of this part already is approaching the second level,

TABLE 2

Utilization of glucose by the whole brain

AGE	GLUCOSE UTILIZATION IN MGM./100 MGM. BRAIN TISSUE	O_2 UTILIZATION IN $mm.^3$/100 MGM. BRAIN TISSUE	GLUCOSE UTILIZATION IN MICRO EQUIVALENTS/100 MGM. BRAIN TISSUE	O_2 UTILIZATION IN MICRO EQUIVALENTS/100 MGM. BRAIN TISSUE	RATIO OF GLUCOSE O_2 UTILIZATION
1–10 days	0.66	100	22	4.5	5.1
10–30 days	0.94	180	31	8	3.9
Adult	0.86	165	28.5	7.5	3.8

while the pallium and the stem remain at the first level for several days after birth.

The slight drop in metabolism of the pallium and the stem between the 1st and 4th day is probably due to the hydration that occurs at this time. Donaldson and Hatai (5) reported that the amount of dry material of the brain falls from 12.6 per cent to 11.9 per cent during the first 4 days of life. This indicates that during that period about 60 mgm. of water have been added to each gram of brain tissue. The addition of such considerable amounts of non-respiring material may account for the drop in oxygen uptake. Although this decrease in solid material also occurs in the medulla, the rise in metabolism in that part no doubt masks the drop to be expected from hydration. Similarly the fact that the drop in the pallium is greater than that of the stem may indicate that the rise in the oxygen uptake begins earlier in the stem than in the pallium.

It is interesting to note that the metabolisms of the various parts reach their peaks in the same order as the transition of the first into the second level takes place, namely, first the medulla (end of the third week), then the stem (end of the fifth week) and finally the pallium (seventh week).

The rise in metabolism coincides with the beginning of myelinization of the

rat's brain (6, 7, 8). This process continues with great intensity until about the 6th week of life (7). It is remarkable that notwithstanding the deposition of considerable amounts of poorly respiring myelin (9) the respiration of the rat brain almost doubles during that time. It might be assumed that the energy required for myelinization and the many other changes occurring during the first month of life (7) results in the high metabolism found during this period. However, the fact that the oxygen consumption does not fall off to the same extent as the reduction of the formation of myelin and other substances, but remains, at least in the stem and pallium, at a continuous high level forestalls this conclusion. The order in which the brain parts start their transition from the first into the second level, and in which they reach their peaks, is the same as the order in which they start functioning. It seems likely, therefore, that the transition is correlated with the beginning of function of the brain parts.

The high glycolytic activity found during the first week of life is of considerable interest in view of the high resistance that newborn animals show to oxygen lack and circulatory arrest (10). It has been suggested (11) that newborn animals can obtain anaerobic energy through glycolysis to a greater extent than the adults.

SUMMARY

1. The oxygen consumption and glucose utilization of excised rat brain were determined at different ages. Three distinct levels in the respiration of the brain can be distinguished. The lowest level which occurs during the first week of life is characterized by a low oxygen and glucose utilization but a high glycolytic activity. The highest level occurs between the 4th and 7th week and is characterized by a high glucose and oxygen utilization but a decrease in glycolytic activity. The adult level is reached at about the 20th week and is somewhat lower than the second level.

2. The order in the rate at which the various parts of the brain take up oxygen changes during development. At birth the medulla has the highest respiration, followed by stem and pallium; in the adult, this order is reversed.

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AN ACID-NEUROHUMORAL MECHANISM OF NERVE CELL ACTIVATION[1]

ROBERT GESELL, CHARLES R. BRASSFIELD AND MARY ALICE HAMILTON

From the Department of Physiology, University of Michigan, Ann Arbor

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The observations reported in this paper present an unlooked for variation of the phenomenon of after-discharge which promises broad application in the field of neurophysiology. This modification was fortuitously revealed in the after-hyperpnea produced by faradic stimulation of Hering's nerve and of the cutaneo-sensory saphenous nerve of the dog. (Morphine urethane anesthesia and both cervical vagi cut.)

Originally the purpose of this experiment was to compare the phenomenon of after-discharge in a respiratory reflex arc with that of the spinal reflex arcs, so well established by Sherrington and his co-workers (1906, 1932). It appeared to us that a demonstrable basic similarity of these two forms of motor integration would permit a more intimate application of the great store of knowledge on spinal integration and thus enhance our understanding of the respiratory act.

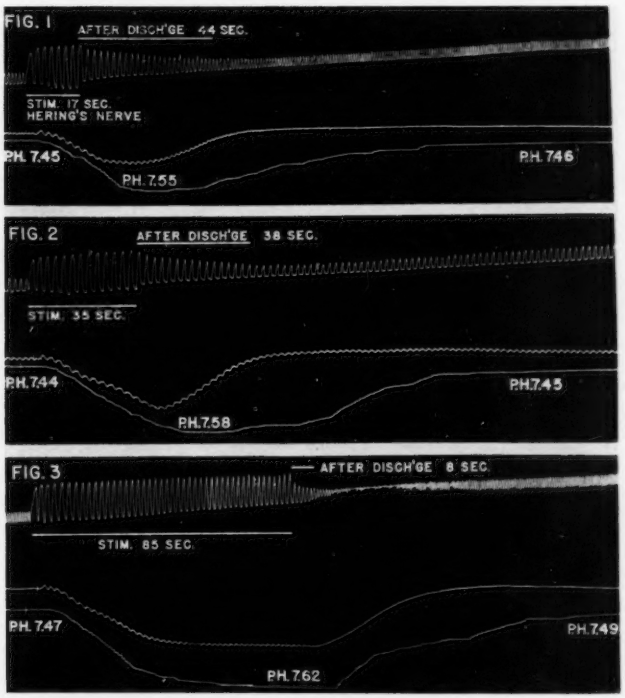
It was to be expected that the reflexogenic hyperpnea elicited by faradic stimulation of chemoceptive and sensory cutaneous afferents would overventilate the blood and tissues and produce a general drop in the hydrogen ion concentration. A continuous record of the changes in cH of the circulating blood (glass electrode in the path of the carotid flow, see Brassfield, 1936) was, therefore, indicated to show the extent and progress of these changes; for a reduction of cH or of CO_2 pressure might, in accordance with the accepted stimulating action of increased cH, be expected to oppose the after-action of reflexogenic stimulation and abbreviate the after-discharge. Therefore, if hyperpnea were found to continue after artificial stimulation had ceased, i.e., during a reflexogenically established hypocapnia and hypo-acidity, it would show in a most decisive way the tendency of nerve cells to continue to discharge in the absence of the original impinging excitatory impulses. This tendency is clearly revealed for the respiratory reflex arc in figure 1. Though the arterial pH rose from 7.44 to 7.55, hyperpnea nevertheless continued for approximately 45 seconds after stimulation of Hering's nerve had ended.

Our main purpose—that of showing a marked similarity of after-discharge in the respiratory and spinal reflexes (see Gesell, 1940)—having been served the problem was for the time being dropped. Only later, on comparing observations on the effects of varying periods of stimulation was it indicated that a more significant principle had been uncovered (see figs. 1, 2 and 3). In figure 1, where stimulation lasted only 17 seconds the phenomenon of after-hyperpnea persisted for a period of 44 seconds. When stimulation was increased to 35 seconds the after-discharge shortened to 38 seconds. And finally when stimula-

[1] Preliminary Report—Univ. Hosp. Bull. (Michigan) 7: 94, 1941.

tion was markedly prolonged to 85 seconds the after-hyperpnea lasted only the short period of 8 seconds.

Such striking inverse relation of after-discharge to stimulation stands in marked contrast to the firmly established findings of Sherrington (1906, 1932) in which spinal reflex after-discharge varies directly with the amount of stimulation. The absence of hyperventilation in the experiments of Sherrington seems to be the critical difference involved and very pointedly indicates a most important rôle of cH in neurophysiology. How then does acidity modify the

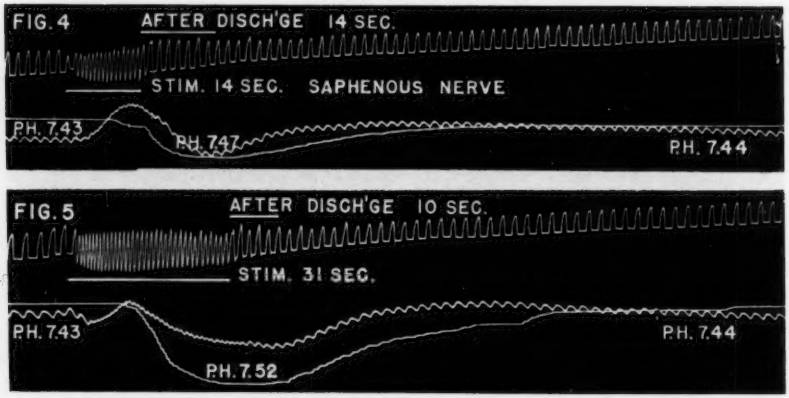


Figs. 1, 2 and 3. An inverse relation of the duration of after-discharge to the duration of faradic stimulation of Hering's nerve. Sherrington's criterion of the return of contraction to the original intensity is employed to determine the duration of the after-discharge.

activity of nerve cells? One possible explanation is that already advanced by Gesell, Lapides and Levin (1940) for the respiratory center, according to which nerve cell activity is a resultant of two forms of stimulation: 1, the direct action of increasing cH upon the nerve cell itself, plus 2, the stimulating action of nerve impulses. Hyperventilation would thus theoretically subtract a fraction of the direct stimulating action of cH from the sum-total of 1 + 2. Gesell, Lapides and Levin (1940) offer a simple electrotonic mechanism by which the addition and subtraction of 1 and 2 may be accomplished.

Without suggesting the elimination of the direct stimulating action of cH on nerve cells as an integrating force we see potent reasons for consideration of an additional mechanism in which increasing cH exerts an indirect stimulating control. On the assumption that nerve cells within the brain are reflexogenically excited by a physiological deposition of acetylcholine (Dale, 1935), as is now recognized for the outlying ganglia, the instability of acetylcholine in alkaline solution repeatedly observed by Dale and his co-workers calls for our attention. In figure 1 where the stimulation of Hering's nerve is shortest and the increase of pH the smallest, the after-discharge is longest. In figure 2 where the increase in pH is somewhat greater the duration of the after-discharge is shorter. In figure 3 where the pH rises to the highest level the after-discharge is shortest.

Granting that the instability of physiologically deposited acetylcholine increases in an increasingly alkaline physiological media we are faced with a new



Figs. 4 and 5. An inverse relation of the duration of after-discharge to the duration of faradic stimulation of the saphenous nerve.

and simple mechanism of an acid-neurohumoral control of nerve cell activity and incidentally of the control of respiration. *By controlling the life span of a single deposit of acetylcholine, acidity thus determines the value (the duration and intensity of action) of that corresponding impulse. By virtue of the same principle acidity would determine the sum-total of existent acetylcholine deposited by the myriads of impinging signals, and thereby the sum-total of stimulation at any given moment and the duration of after-discharge following cessation of inflowing impulses.* That in short is our theory of the acid-neurohumoral mechanism of gradation of nerve cell activity.

Variations in duration of stimulations of the saphenous nerve though eliciting a hyperpnea in which accelerated rhythm predominated produced the same type of changes in the after-discharge described for Hering's nerve (see figs. 4 and 5). Stimulation of only 14 seconds in figure 4 increased the pH from 7.43

to 7.47 and produced an after-discharge which lasted approximately 14 seconds whereas in figure 5 where stimulation continued for 31 seconds and raised the pH from 7.43 to 7.52 the after-discharge endured for only 10 seconds.

Such striking similarity of results obtained on nerves of such diversity of specialization and function is of interest in suggesting that the acid-neurohumoral mechanism is not confined to afferent impulses solely related to the respiratory act in which the importance of cH as a regulatory factor has long been recognized. In fact recent experiments indicate the existence of an acid-neurohumoral mechanism at all central and peripheral junctions wherever acetylcholine is physiologically deposited (Gesell, Brassfield and Hansen, 1941; Hansen, Worzniak and Gesell, 1942; Brassfield and Gesell, 1942; Mason and Gesell, 1942; Gesell, Brassfield and Hansen, 1942). Thus we have discovered in a purely accidental way a mechanism which Winder (1938) and Worzniak and Gesell (1938) had sought by a direct approach. Winder had attempted to show the interaction of acidity and acetylcholine in the activity of the carotid body but failing to obtain conclusive evidence tentatively dropped the experiments on the chemoceptors. Worzniak and Gesell studied the possibilities of an acid-neurohumoral mechanism of nerve cell activation in the respiratory center. Although several procedures gave indication of a complementary action between acid and acetylcholine their results were also regarded as suggestive rather than conclusive. Only in retrospect do these findings appear more substantial than was realized.

It is strange that a mechanism which, in the light of increasing evidence, seems so obvious should have eluded recognition so long. The instability of acetylcholine in alkaline solution was well-known. So was its destruction by cholinesterase. Furthermore, Plattner, Galehr and Kodera (1928) showed that the rate of destruction of acetylcholine by cholinesterase in blood serum or in a suspension of red blood cells increased with increasing alkalinity in the range of pH 5.6 to pH 8.2. Feldberg and Schriever (1936) found that asphyxia led to the appearance of acetylcholine in the cerebrospinal fluid of a lightly eserinized animal but they make no mention of the undoubted increase of cH of the brain and the preservative action which this might have had. They conclude their paper as follows "We have as yet no data which would warrant speculation as to the relation between this output of acetylcholine after adrenaline and asphyxia and any functional changes in the nerve centers". Andrus (1924) finds that the inhibitory action of acetylcholine on the rabbit's auricle is greater at a pH of 7.0 than at a pH of 8. Stimulation of the vagus nerve was also more effective at a pH 7.0. Clark (1927) describes a decreasing effectiveness of acetylcholine on the ventricle of the frog with increasing alkalinity.

The slowness of destruction of acetylcholine, generally regarded as the strongest argument against the neuro-humoral theory (see Eccles, 1936) must now be reconsidered in a new light for it seems highly probable that fluctuations in the rate of disappearance of acetylcholine is part and parcel of an elastic mechanism of gradation of nerve cell activity.

SUMMARY

The duration of respiratory after-discharges following faradic stimulations of either Hering's nerve or the cutaneo-sensory saphenous nerve varied inversely with the duration of stimulation. Such findings are contrary to the direct relation of after-discharge to stimulation as found by Sherrington in spinal reflexes. These differences in respiratory and spinal after-discharges were attributed to the alkalinizing action of the reflexogenic hyperpnea. Since acetylcholine in alkaline media and in the presence of cholinesterase is very unstable it has been proposed that variations in cH may determine the duration of the life span of each deposit of acetylcholine produced by each impulse impinging on a nerve cell. Variations in the life span of individual deposits would in turn determine the rate and amount of accumulation of acetylcholine at nerve cells under bombardment, as well as the rate at which accumulated acetylcholine disappeared when bombardment ceased. Prolonged hyperpneas which turned the tissues more alkaline than shorter hyperpneas might, therefore, be followed by shorter periods of after-hyperpnea. Findings conform with theory. It has been suggested that the control of the longevity of acetylcholine deposits by variations in cH may prove to have an important rôle in neurophysiology.

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THE SPONTANEOUS ACTIVITY AND FOOD INTAKE OF RATS WITH HYPOTHALAMIC LESIONS[1]

A. W. HETHERINGTON AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School, Chicago, Illinois

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Bailey and Bremer (1921) speak of "apathy" in their two obese dogs which had hypothalamic lesions, and several of Brown's (1923) fat dogs became "sluggish." Smith (1930), evidently referring to certain early postoperative symptoms, stated that rats which had received an injection of chromic acid into the hypophysis, presumably injuring the hypothalamus, like other rats with hypothalamic lesions, displayed periods of quiet interspersed with periods of excitement. Speaking also of rather early postoperative activity, Krieg (1938) mentioned, among other manifestations, an "emotionally hyperactive state," and "depression."

Although these and similar incidental references have been made by various workers to the behavior and activity of animals with damage to the tuber cinereum, apparently with but one exception (Richter, 1930) no one had tried until very recently to compare quantitatively the gross activity spontaneously exhibited by such animals with preoperative or normal values. Richter produced diabetes insipidus in seven rats by stabbing a short narrow blade through the sphenoid bone at a point he judged to be just rostral to the hypophysis. As yet the nature of these hypothalamic lesions, if indeed such they were, has not been described; but they probably occupied an area in the neighborhood of the median eminence. The result of this procedure was found to be essentially negative. Rats which had run well before the operation continued to do so afterward, and the poor runners remained inactive.

Food intake of these animals, Richter found, was likewise unchanged. Of course, none of the rats observed in these experiments became obese and it may be assumed that they did not bear the sort of hypothalamic lesions attributed by Hetherington and Ranson (1940) with the capacity to cause adiposity.

Other investigators, however, have reported a contrary finding. Keller, Hare and D'Amour (1933) and Keller and Noble (1935) observed enhanced food intake and a tendency to adiposity both in dogs with hypothalamic lesions and in dogs with a variety of pituitary injuries. Ranson, Fisher and Ingram (1938) noticed that one of their monkeys with hypothalamic lesions increased rapidly in weight and displayed marked polyphagia.

Finally in two simultaneous preliminary reports Tepperman, Brobeck and Long (1941) and Hetherington (1941) have added the most recent information on the subject. The former group found that certain rats with hypothalamic lesions became extremely obese and consumed approximately twice as much food

[1] Aided by a grant from the Committee on Research in Endocrinology of the National Research Council.

as litter-mate controls. Hetherington, on the other hand, observed a group of operated rats which did not eat more than their controls, but which, nevertheless, in some instances did grow fat. The latter's animals were observed, in addition, to engage in a great deal less spontaneous running activity than did the controls.

The following experiments contain an elaboration of the results reported by Hetherington (1941) and a considerable amount of new material which has been added since that time.

METHODS. The present series numbers 18 male rats, 11 operated animals and 7 controls, run in groups of 6. Each group was made up of litter-mates. One control and one operated animal failed to survive the full length of time covered by the experiments and are not included in the results. Hypothalamic lesions were placed in the operated rats by the method described by Hetherington and Ranson (1940).[2]

For study of spontaneous running activity the type of cage, with slight modifications, described by Richter and Wang (1926) was used. The modifications were as follows: The living compartment is considerably smaller on these cages, being 3 inches wide by 5 inches high by 6 inches long. The revolving drum is a little larger, having a diameter of 15 inches. It is balanced in order to enable it to be stopped at any position, and revolves so easily that a weight of less than half a gram at the periphery will cause it to turn.

The cages were not kept in an air-conditioned room; consequently humidity undoubtedly varied over a considerable range. The temperature of the room, however, was kept within the range between 75° to 78°F. (Animals with hypothalamic lesions generally require a warmer room than normal for maintained good health.) They were subjected to 12 hours of illumination and 12 hours of darkness per day, the lights being controlled by a Tork electric clock.

The food supply was altered from group to group in the following manner: The first group received nothing but Rockland Rat Ration pellets for the first 5 weeks of the experiment. During the final 3 weeks the pellets were ground and moistened with water. The other two groups received a mixture made of 37 per cent ground Rockland rat pellets, 18 per cent ground dry white bread, and 45 per cent raw whole milk by weight. Needless to say, samples of this diet (as well as of the pellets first used) were dried daily to determine their moisture content; and all figures given in the results are dry weights calculated from the dried food residue collected at the end of each 24-hour period.

To determine the influence of activity upon food intake the second two groups were kept part of the time in small cubical living cages ($7\frac{1}{2}$ in. on a side), and the remainder of the time in the activity cages. One group spent the first 6 post-operative weeks in the activity cages and the next 4 or 5 weeks in the stationary cages. The process was reversed with the other group, the first 5 weeks being spent in the ordinary living cages and the final 4 weeks in the activity cages.

RESULTS. The lesions found in the hypothalami of the operated rats need not be described in detail at this time. It is sufficient to say that they conformed

[2] For the Evipal anesthetic used in the operations we are indebted to Dr. J. J. Kuhn of the Winthrop Chemical Co.

in a general way with the lesions found in a former series (Hetherington and Ranson, 1940). As before, lesions producing the higher degrees of adiposity in this series lay on both sides in the region of the ventromedial hypothalamic nucleus and its immediate cellular environs. Lesions failing to precipitate the syndrome, or causing it to only a minor degree were rather markedly asymmetrical, not near enough to the base, or for some other reason inadequate.

When the experiment with each group of animals was terminated, the rats were weighed, anesthetized, and their body length (nose-anus) measured. They

TABLE 1

Table summarizing the data on age, weight, and body length of the rats at the time of the termination of the experiment

RAT NO.	OP. OR CON.	AGE	WEIGHT	NOSE–ANUS LENGTH	$W^{\frac{1}{3}}/L$	DEGREE OF ADIPOSITY
		days	*grams*	*cm.*		
Rf-1	C	133	385	24.7	0.294	
Rf-2	O	133	380	23.8	0.304	+
Rf-3	C	136	373	24.3	0.296	
Rf-4	O	133	342	23.1	0.303	?*
Rf-5	O	136	347	20.5	0.342	+++
Rf-6	O	136	325	23.1	0.297	−
Rf-7	O	172	485	23.8	0.330	+++
Rf-8	C	172	413	25.1	0.297	
Rf-9	C	157	371	23.9	0.300	
Rf-10	O	157	473	24.3	0.321	+++
Rf-11	O	157	449	23.4	0.327	++
Rf-12	C	died				
Rf-13	O	153	413	22.9	0.325	+++
Rf-14	O	died				
Rf-15	C	153	323	23.1	0.297	
Rf-16	C	153	335	23.0	0.302	
Rf-17	O	153	349	23.5	0.299	−
Rf-18	O	153	331	22.5	0.307	?

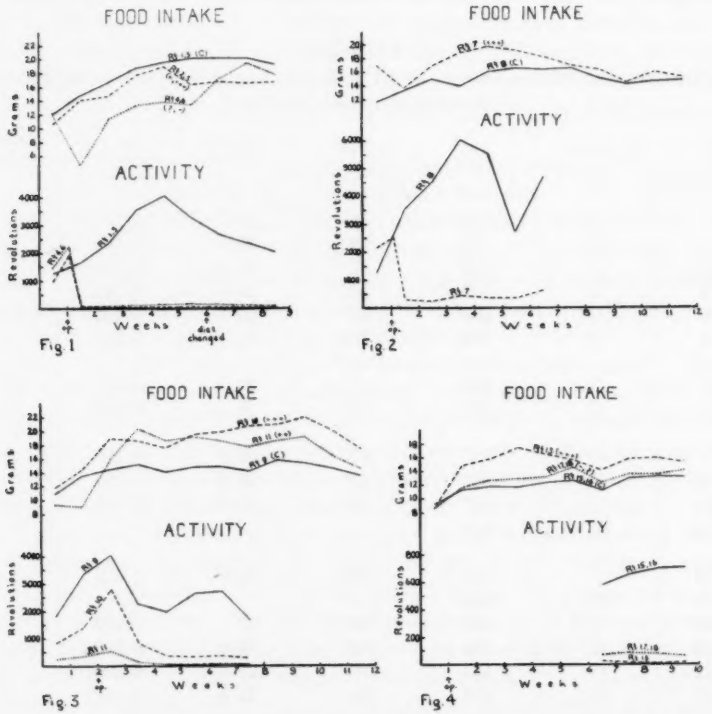
* Indicates presence of adiposity doubted.

were not always immediately killed. The data obtained at this time are summarized in the table, which shows age, weight, and body length, and several indices of degree of adiposity which will now be explained.

The formula $W^{\frac{1}{3}}/L$, expressing the ratio of the cube root of the body weight in grams to the body length in centimeters, was borrowed from Lee (1929), who was interested in the expression of metabolic results for white rats. Following Cowgill and Drabkin (1927), who applied the formula to the dog, Lee used this "nutritive correction factor" to indicate the nutritive state observed in an individual animal.

In the table opposite the weight-length ratio of each operated rat is placed a

symbol, either a minus-sign, or one, two, or three plus-signs, which represents a visual estimate of the degree of an animal's adiposity. This estimate was based on a careful inspection and comparison of the rat with its control, and signifies that the animal was judged either not to be fat, or to be slightly, moderately, or markedly obese. The two classifications of adiposity have not always given results completely consistent with each other, yet they do not in any case fundamentally disagree.



Figs. 1–4. Records of food intake and spontaneous activity (running) of obese and nonobese rats with hypothalamic lesions, and of their normal litter-mate controls. In figure 1 the change in diet at the end of the fifth postoperative week should be noticed. In figure 4 note the vertical scale for activity is different from that in the first 3 figures.

No attempt has been made to divide the weight-length ratios arbitrarily into groups. Inspection of the table will reveal, however, that no control rat exhibits a ratio above 0.302; the average of the ratios of the 6 normal males is 0.298. Determined for a much longer series of normal males used in other experiments the figure is slightly lower—about 0.293—with an upper limit to the range of normal values, as here, at about 0.302. No male which was considered definitely obese has a ratio below 0.304, and the fatter animals have ratios above 0.320.

All data secured from the activity cage experiments and from determinations of food intake are summarized graphically in figures 1 to 4.

The first 6 animals were placed in activity cages at the age of 6 weeks, and 4 of them were operated 2 weeks later. Two of the operated rats in a matter of 4 to 6 weeks showed an unmistakable degree of adiposity, while the other 2 retained an essentially normal appearance. In figure 1 the mean daily food consumption of each rat for any given week is averaged together with the mean daily food consumption of the other fat rat for the same week, and the average of the means is plotted on the graph as a single point. The same procedure is followed with the figures for daily activity, and is applied to the corresponding determinations for the pair of controls and the pair of nonobese operated animals. This has been done purely to reduce for the sake of simplicity and clarity the number of lines on the graph. With particular reference to the upper set of lines in figure 1 the point should be stressed at this time that the paired representation of food intake does not conceal an overlapping of the data of the normal and obese rats. Neither of the fat animals ate as large an amount of food as either of their control litter-mates.

As was mentioned before, the diet of these animals consisted during the first 5 postoperative weeks of whole Rockland rat pellets. On this diet the 2 nonobese rats maintained a considerably lower level of food intake than the others until the final 3 weeks of the experiment, when grinding and moistening of the food pellets (with water) seemed to exert a favorable influence upon their food consumption. The change apparently did not induce the 2 obese and 2 normal animals to alter their eating habits.

With regard to spontaneous running activity of the animals, the graph (fig. 1) speaks for itself. In this set of animals, and indeed in all the others as well (figs. 2–4), the trend is clear. Rats with large hypothalamic lesions in the region dealt with here evidently indulge in a great deal less running activity than do the majority of normal animals, or than they themselves did previous to the placing of the lesions. The change is striking and practically immediate, occurring within one or two days after the operation. There is a suggestion that the obese animals are even more inactive than those which do not grow fat (figs. 1, 4), but in view of the small number of animals tested, the difference might not be significant.

Figures 1 to 3 illustrate a state of hyperactivity which usually appears during the acute postoperative stage. Often rats with large lesions in this region of the hypothalamus will run almost continuously in an automatic, almost frenzied fashion for several hours after they awake from the anesthetic. This period may be succeeded by another lasting several days when the animals will seem to be stuporous, but will respond with exaggerated violence to slight tactile stimuli. The phenomenon was noticed many times in this laboratory, even long before the work on activity was begun. After the acute phases of the postoperative period are past the rats are rather lethargic, though this characteristic usually does not appear to partake of somnolence or a lack of alertness. They are, in fact, generally somewhat irritable and excitable for a number of weeks. With handling the rats after a time often lose this touchiness to a certain extent. (It should be mentioned, in passing, that more recently operated rats with lesions in the caudal hypothalamus have displayed neither the acute hyperactivity nor

the later hyperirritability of the rats just described. As a matter of fact, they tend to be rather amiable and more passive than normals. Even rats with the more rostrally located injuries will display the symptoms to a much slighter degree if the lesions in question are small.)

In a preceding section it was explained that the second and third groups of animals were fed a mixture of ground Rockland rat pellets, ground dry white bread, and raw whole milk. Food intake on this diet may now be considered.

Figure 2 shows how one operated rat which was accustomed to eating somewhat more than its control even before placing of the lesions, maintained, or even widened the margin between its food intake and that of its control after operation. After the animals were removed from the activity cages the food intake of the pair became more nearly equal.

In figures 3 and 4 is to be found a much clearer demonstration of the fact that these obese rats with hypothalamic lesions under certain circumstances will consume a good deal more food than do their litter-mate controls.

The final experiment, consisting of a comparison of the food intake of the animals during their sojourn in the activity cages, where it seems likely more exercise is taken by normal rats, with the food intake observed during the period spent in ordinary cages, has been rather inconclusive. In figure 2 there appears to be a slight decline in the food intake of both the normal and the obese rats after removal of the animals from activity cages. There furthermore seems to be a smaller difference between the intakes of the 2 rats, for which the more rapid descent of the curve for the fat animal is responsible. In figure 4 (where again the method of averaged daily means for the pair of controls and the pair of non-obese operated animals is used) is shown a small increase in food intake which occurred when the animals were placed in activity cages.

The rats represented in figure 3, however, reacted at first—apparently somewhat illogically—with a slightly increased food consumption after being transferred from their activity cages. Later these animals, too, decreased intake, and again the margin between the obese and the control rats narrowed because of the more rapid decline in intake by the fat rats. In any case, the changes in food intake which may be associated with the changes in spontaneous activity assumed here are not at all of the same order of magnitude as those which occurred in some of the obese rats, or even in the normal controls during the phase of rapid growth.

Discussion. The phenomenon of adiposity is often regarded as a problem involving as causes either lack of exercise or over-eating, or a combination of the two. Doubtless in many cases these simple and easily understood factors may be an adequate explanation for excessive weight. In many other cases, however, as for instance in hypothyroid obesity, uncontrollable adiposity following pregnancy, and Cushing's syndrome, some more fundamental cause, intimately tied in with pathological physiology must be sought. (For critical analyses of recent theories regarding "exogenous" and "endogenous" obesity, see reviews by Wilder, 1938, and Bauer, 1941.)

Similarly, in the case of the experimental hypothalamic obesity being investi-

gated here, the most obvious explanations, decreased activity and augmented food consumption, were first taken up for examination, with the results that have been cited. It is true that these animals exercise a great deal less than normal rats. Yet animals which have somewhat similar lesions but which do not grow obese also indulge in much less spontaneous activity, though perhaps not as little as fat rats. It is also true that under certain circumstances, as when the food is softer, easier to eat, and possibly more palatable, the obese animals will consume excessively large amounts of it. This observation does not alter the fact, however, that these rats will also grow fat—though in all likelihood more slowly—even when food intake is limited to an amount equal to or even a little smaller than that of normal litter-mates.

When the simultaneous reports of preliminary work by Tepperman, Brobeck and Long (1941) and Hetherington (1941) appeared, the former group (who had done little or no work on activity) emphasized the high food intake of their animals; whereas the latter was more impressed by the tremendously decreased activity of the obese rats. Insistence upon the primary importance of either viewpoint would in all probability represent over-simplification of the problem, and this for at least two reasons.

In the first place, the two factors are complementary in their effect upon body weight. Both would tend to increase it. A very sedentary life, combined with a high caloric intake would seem to be an ideal combination for building up a thick panniculus adiposus.

Secondly, these two factors may be only symptomatic, and not fundamental. It is not difficult to imagine, for example, a condition of hidden cellular semi-starvation caused by a lack of easily utilizable energy-producing material, which would soon tend to force the body either to increase its general food intake, or to cut down its energy expenditure, or both. In this connection, it would be of great interest to determine whether these animals exhibit a preference for any particular class (chemically speaking) of foods.

It should be clearly realized that the apparent reluctance of the bodies of these fat rats to utilize their tremendous stores of fat is only relative and not absolute. Brobeck (1941) has stated that his fat rats can be fasted down to a normal body weight; and it has been noticed many times in this laboratory that a rat can stop eating and lose up to a third or more of its body weight before it recovers its appetite. These animals can, therefore, use fat as a source of energy if necessary, though perhaps at a low level of efficiency. The concept which comes to mind is one much like that expressed by Thomson (1938), who, discussing a somewhat similar matter, speculated upon the varying availability to the cell of different substrates of energy-furnishing material.

Evidence for a more basic disorganization of the physiological economy of these animals is not voluminous, but it is suggestive. To begin with, Hetherington and Weil (1940) showed that there was a pronounced deficit in the total body phosphorus of the obese rats with hypothalamic lesions. Although there was a co-existing calcium deficiency as well, the calcium-phosphorus ratio was irregularly altered in such a way that it was believed other phosphorus containing

materials besides bone had suffered. The importance of phosphorus in fat metabolism, particularly fat transport, and as a constituent of numerous physiologically important organic compounds in the body hardly requires elaboration.

In addition, Tepperman, Brobeck and Long found that the basal oxygen consumption of obese rats which had been either fasted for a long time, or pair-fed with their controls was low compared to the normals; while R.Q. determinations in the absorptive state were higher. Daily creatinine excretion was high in their rats, and carbohydrate metabolism in some instances appeared to be affected. Grafe and Grünthal (1929) found lowered B.M.R.'s in obese dogs which were supposed to have hypothalamic lesions.

It is argument along these lines which has suggested the imperative need for further fundamental physiological and biochemical research on rats displaying hypothalamic obesity. The ease with which the syndrome can be produced in the rat, and the wide variety of physiological techniques which can readily be applied to the animal make it an ideal subject for investigation.

SUMMARY

The spontaneous running activity both before and after operation of 10 rats with hypothalamic lesions frequently causing adiposity and of 6 normal litter-mate controls has been investigated. In addition the food intake of all the animals has been measured, in some cases both in activity cages and in ordinary cages.

It has been found that animals having bilateral lesions in the medial hypothalamus in the region in and around the ventromedial hypothalamic nucleus tend to indulge in much less spontaneous running than do the majority of normal controls, or than was exhibited preoperatively.

Food consumption of the obese operated animals may greatly exceed the intake of the normal litter-mate controls or may not exceed it at all, depending upon the nature of the food supplied. A soft palatable diet encourages maximum consumption, and a hard dry pellet diet apparently discourages high intake. Animals probably grow obese more rapidly on the former type of diet. The idea is suggested that the obese animal's efforts to increase food intake and cut down energy expenditure are indicative of a partial inability on the part of its physiological mechanism to metabolize easily all of its available food stores.

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MUSCLE TREMORS AND THE DEVELOPMENT OF TEMPERATURE REGULATION IN BIRDS

EUGENE P. ODUM

From the Department of Zoölogy, University of Georgia, Athens

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The term muscle tremors, as here used, refers to the coördinated, semi-rhythmic partial muscle contractions or changes in muscle tone which, when pronounced, produce visible shivering. It is generally recognized that tremors are important in temperature regulation of homoiothermic animals. However, it is still a matter of debate as to whether variations in muscle activity alone are sufficient to account for increased metabolism accompanying a decrease in environmental temperature, or whether extra-muscular (i.e., "chemical regulatory") mechanisms are also involved (1). In connection with recent studies (2) on the heart rate of small birds utilizing a specially designed apparatus, the cardio-vibrometer, data were obtained which indicated a close correlation between ontogenetic development of temperature control and the development of definite, coördinated muscle tremors.

METHODS. A description of the cardio-vibrometer[1] has been recently published (2, 3). In the experiments with young birds, nestlings of known age were removed from their natural nest and placed in a simulated nest attached to the sensitive piezo-electric crystal (the pickup device); in this way vibrations whether due to heart beat, breathing, or skeletal muscle activity could be recorded (with crystal driven pen writing on moving paper) with a minimum of disturbance to the bird. The amplifier and recorder were designed to record low frequency vibrations up to 60 per second. From the graphic records thus obtained tremors could usually be distinguished from heart beat, breathing, or other recorded movements; furthermore, tremors could be detected in this way when intensity was not great enough to produce visible shivering. All records were made under standard ("basal") conditions in which birds were maintained in darkness, 2 to 4 hours after last feeding, and at a controlled temperature. Usually the pickup and the bird in the sound-proofed temperature chamber were placed in a different room from that containing the recorder and observer. Nestlings of the house wren (*Troglodytes aedon*) and the black-capped chickadee (*Penthestes atricapillus*) were largely used, although a few experiments were run on precocial species including the ring-necked pheasant (*Phasianus colchicus*), common tern (*Sterna hirundo*) and the domestic fowl.

RESULTS. Figure 1 is a reproduction of actual cardio-vibrometer records (vibrograms) of two ages of nestling house wrens when resting quietly at standard conditions. The house wren, like other altricial birds, recapitulates during the short period of its nest life the evolutionary development from the poikilother-

[1] The construction of a new and improved apparatus has been made possible by recent grants from the A.A.A.S. and the University Research Center of Georgia.

mic physiology of reptiles to the homoiothermic physiology of birds, the most rapid changes taking place between 4 and 12 days after hatching (Kendeigh, 4). In figure 1, A, B, C are typical records of newly hatched (0-day) nestlings at progressively decreasing temperatures (95°, 80°, 70°F. or 35°, 26.7°, 21.1°C.).

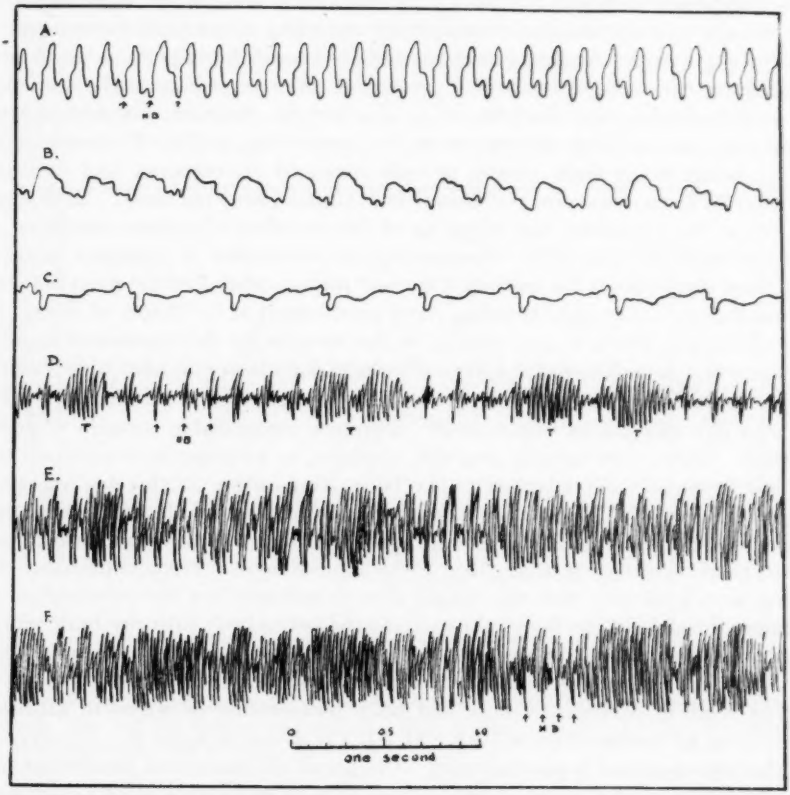

Fig. 1. Vibrograms of nestling house wrens illustrating the effect of decreasing air temperature on recently hatched (0-day), poikilothermic nestlings as compared with 12-day, homoiothermic nestlings. Heart beat(HB) and muscle tremors(T) are indicated. A, B, C, 0-day nestlings at air temperatures of 95°, 80°, 70°F. (35°, 26.7°, 21.1°C.) respectively. Heart rates are 404, 227, and 116 per minute. D, E, F, 12-day nestlings at 95°, 80°, 70°F. respectively; heart rates are 418, 520, and 690 per minute. Note: The difference in the form of the tracings is due to difference in position of birds on the pickup crystal; thus, newly hatched nestlings rest on their chests producing a record similar to "apex beat" of human cardiology.

As would be expected of a cold-blooded animal the rate of heart beat as well as the amplitude decreased as the temperature dropped. In 12-day nestlings (fig. 1, D, E, F), however, the heart rate increased markedly with the same drop in air temperature as would be expected in a warm-blooded animal. Further-

more, pronounced tremors were present which increased with the decrease in air temperature until at 70°F. tremors were nearly continuous and almost obscured completely the recording of the heart beat (fig. 1 F). In striking contrast, no such coördinated tremors were detected in the cold-blooded nestlings; thus, in figure 1 A, B, C, the pen line is smooth, no high frequency (relatively) vibrations interfering with the recording of the heart movements.

The way in which tremors first developed is interesting and perhaps significant. At higher temperatures tremors, if present at all, were often periodic occurring in short periods, sometimes less than a second in duration, alternating with longer periods in which no tremors were recorded (fig. 1 D). With a decrease in air temperature these tremor periods increased in frequency and duration until at 70°F. tremors were continuous in 9, 12 and 15 day old birds. Even then, however, the frequency and intensity of the recorded vibrations continued to vary periodically (fig. 1 F). Tremors appear to develop in ontogeny in much the same way often being restricted to short periods when first detected between 3 and 6 days of age and becoming more pronounced at 9, 12 and 15 days. In the chickadee, which is very similar to the wren in its developmental history, tremors were first detected at 4 days after hatching which coincided with the first appreciable rise of the body temperature above that of the environment. With most 4-day chickadees tested at 70° definitely recognizable tremors were recorded. These were usually periodic, sporadic, or irregular in occurrence and of low frequency and intensity. The body temperature at this age averaged 2° to 4°F. above 70°F. In most 3-day nestlings, on the other hand, recognizable tremors could not be picked up by the apparatus, and the body temperature was equal to or only slightly above that of the environment. Tremors probably develop more gradually than this would seem to indicate, but the coördination of tremors throughout the body (thus setting the entire body into rhythmic vibration and producing an unmistakable graphic record) seem to occur coincident with the first indications of the development of temperature control.

The relation between tremors and body temperature of wrens of different ages at an air temperature of 70°F. (21.1°C.) is shown in figure 2. A curve for feather development is also included. Counts of the number of penstrokes (on graphic records) definitely resulting from tremor movement were taken as a means of quantitative measurement of tremors. Samples were taken at 5-second intervals over many feet of records so that periods without tremors would be averaged in with tremor periods in proportion to their occurrence. Thus, both the duration (ranging from 0 to 100 per cent) and the actual frequency of the coördinated "firing" of muscle units during active periods (usually ranging between 30 and 40 per second) were taken into account in the average figures plotted in figure 2.

Apparently, the control of heat loss as indicated by the feather development lags behind the development of the muscle tremor heat production mechanism. Thus, at 9 days of age tremors were vigorous but feather development poor and body temperature low (at standard conditions). At 15 days feather development more than doubled and the body temperature was maintained higher

with about the same amount of tremors. Finally, the normal body temperature of the adult is maintained with few or no tremors at this particular temperature. The curve of standard heart rate is roughly similar to that of muscle tremors; heart rate increases to 12 days then decreases in the adult (Odum, 2).

Increase in tremors in nestlings with a decrease in air temperature often occurred with little increase in general activity (i.e., actual movements) although this was not measured quantitatively over long periods. When movements did occur during the recording periods it was very noticeable that tremors stopped or slowed down immediately after and sometimes just before the movement. Accordingly, tremors and movements operate in a complementary

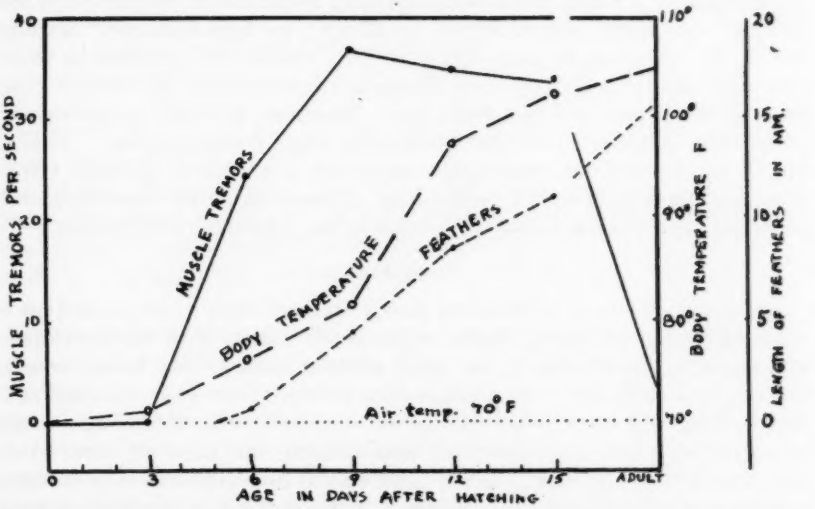

Fig. 2. Relation of muscle tremors, body temperature and feather development in house wrens. See text for further explanation. Body temperature data are from Kendeigh (4) and feather data from Boulton (5).

manner. An anticipatory stopping of tremors preceding a movement was especially striking in records of adult birds incubating on their nests (Odum, 2).

In preliminary experiments on precocial birds, in which temperature control begins to develop before hatching, what appear to be brief tremor periods (producing a record similar to fig. 1 D) have been detected as early as 9 days' incubation in the case of unopened eggs of the ring-necked pheasant at 99°F. (37.2°C.). According to Romanoff (6) this is approximately the period in the development of the domestic fowl when embryo temperature begins to rise above incubation temperature.

Discussion. In making quantitative measurements of these total body tremors from records such as shown in figure 1 a number of variables need to be considered if a complete picture of the response to temperature changes is obtained. As already indicated the relative duration as well as the frequency of

tremors is important. In 12 day wrens, for example, tremors were recorded 12 per cent of the time at 95°, 93 per cent at 80°, and 100 per cent (shivering continually) at 70°F. The frequency of tremors in these small birds is apparently upwards to 30 or 40 per second, considerably more rapid than the shivering rate reported for man (7). Activity as mentioned above is another factor effecting tremors. Still another variable is the amplitude or intensity of the individual tremor contractions. Apparently amplitude increases with an increase in duration and frequency, but this could not be measured quantitatively with the setup used.

These various muscle adjustments produce a very sensitive mechanism in these small birds which seems to be especially responsive at temperatures below thermal neutrality (about 100°F. or 37.8°C. in homoiothermic nestlings). Within the temperature range which we have considered variations in tremors and other muscular activity seem adequate to account for the observed variations in heart rate and metabolic rate. However, at other temperatures or under other conditions different mechanisms might come into play. As Dworkin (7) has pointed out temperature regulation as a whole is probably complex involving many factors and mechanisms, efficient regulation depending on the coördination of various factors by centers in the central nervous system.

SUMMARY

The development of coördinated muscle tremors, such as are picked up and recorded by the cardio-vibrometer, corresponds closely with the development of temperature regulation in the small altricial species,—the house wren and black-capped chickadee. No tremors were recorded from newly hatched and 3-day nestlings which are poikilothermic but were present at all later ages roughly corresponding to the development of homoiothermy and inversely related to the air temperature. At 95°F. (35°C.) (and also at first appearance in ontogeny) tremors were often periodic in occurrence; with a decrease in temperature tremor periods tended to increase in duration and frequency until shivering became continuous. During active periods the frequency of tremors (as indicated by total body vibrations) was 30 to 40 per second. The muscle tremor heat production mechanism apparently develops more rapidly at first than does the control of heat loss as indicated by feather growth. In the precocial pheasant periodic tremors were first detected at 9 days of incubation in the unopened egg at incubation temperature.

The writer is indebted to Dr. S. C. Kendeigh under whose direction these experiments were begun and to the officers of the Edmund Niles Huyck Preserve, Rensselaerville, N. Y., where experiments with the chickadees were carried out.

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SPEED OF RESPONSES OF VARIOUS MUSCLES OF CATS

J. H. WILLS[1]

From the Department of Physiology in the Harvard Medical School

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In a recent paper Rosenblueth, Wills and Hoagland (1941) reported that the sartorius and soleus muscles of cats, when stimulated indirectly, could show two crests in both electrogram and mechanogram. The temporal properties of the two components of soleus were different from those of sartorius. The present work was undertaken to examine this phenomenon in a greater variety of muscles, and also to see whether different nerve-muscle systems display the phasic activity of muscle described by Rosenblueth and Cannon (1940) and Cannon and Rosenblueth (1940). The latter point was studied with reference to a possible parallelism between the speed of a muscle and its properties as regards the several "stages of transmission."

METHODS. Seven muscles were used: diaphragm (Head's slip), gastrocnemius, inferior oblique of eye, plantaris, sartorius, soleus and tibialis anticus. In most cases the cat, anesthetized with dial (Ciba), was the animal used, but in a few of the diaphragm experiments rabbits anesthetized with urethane were the subjects.

For the study of single twitches the muscles were attached to a torsion spring myograph of the Sherrington type. The contractions were isometric. The diaphragm was studied semi-isometrically because of inability to devise any satisfactory method for strict isometric recording from Head's slip.

During prolonged stimulation for registering the phasic properties of the nerve-muscle system, the activity of the muscle was recorded by a writing lever pulling against rubber bands. The magnification was about 10-fold.

Electrical records were made from the muscles simultaneously with the single twitch except for diaphragm and inferior oblique. The leads were silver needles, chlorided when a direct-coupled amplifier was used. The muscle was crushed about 5 mm. below the tie connecting it with the myograph, and one needle was inserted into the tissue between the tie and the crush. The other lead was in normal muscle.

In most of the experiments a capacity-coupled amplifier with relatively small coupling condenser was used, but in a few cases a direct-coupled amplifier was employed. The amplified responses were led to a cathode-ray oscillograph. The image of the oscilloscope screen was photographed on film simultaneously with the myograph tracing, obtained by sending the beam of reflected light from the mirror of the myograph to the back of the film in the camera.

Spread-out records of the spike potentials of the various muscles were obtained by making single standing photographs with the sweep of the cathode-ray

[1] Porter Fellow of the American Physiological Society.

tube at high speed. The sweep was tripped by the stimulus, so that the records start with the shock artifact. The speed of the sweep was calibrated with known sine-wave frequencies.

The stimulating electrodes were shielded silver wires applied to the motor nerve, crushed or cut centrally. The stimuli were condenser shocks rendered diphasic by passage through a transformer. The frequency of stimulation was regulated by a gas-discharge tube (type 885).

RESULTS. Figure 1 shows the simultaneous isometric mechanograms and monophasic electrograms of the various muscles. It will be seen that several, but not all, of the muscles had two peaks in their mechanograms. The times required for the muscles to produce the various tension peaks can be determined

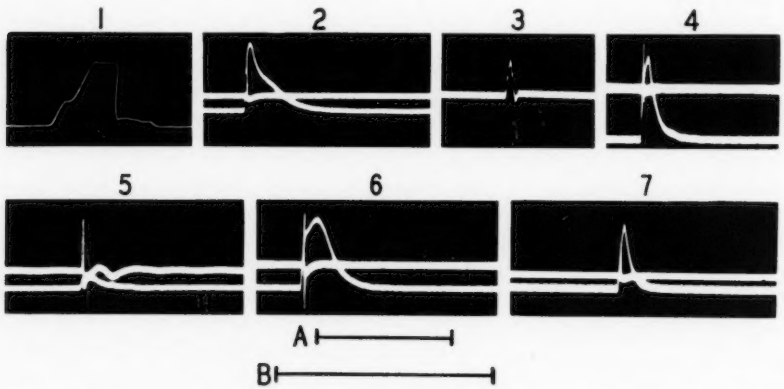

Fig. 1. Simultaneous isometric mechanograms (lower tracings) and monophasic electrograms (upper tracings) of muscles. In the cases of diaphragm and inferior oblique only the myogram is shown. The myogram of diaphragm was not strictly isometric. 1—diaphragm, 2—gastrocnemius, 3—inferior oblique, 4—plantaris, 5—sartorius, 6—soleus and 7—tibialis anticus. Calibrations: A, 1 sec., for diaphragm. B, 1 sec., for all other muscles.

from these records by use of the calibrations for speed of the recording surface. Such values appear in table 1.

Figure 2 consists of the spread-out spikes of the action potential of the various muscles. The lower part of each panel is the calibration of the speed of the sweep. Here also some records have two or more peaks, and some have only one. The times from the beginnings of the action potentials to the various peaks appear in table 1, along with the corresponding times for the mechanograms.

The muscles in table 1 have been arranged vertically according to increasing speed of reaching the major peak of tension. The average times from the beginning of contraction to maximal tension were: diaphragm 480 msec., soleus 77.7 msec., sartorius 28.7 msec., plantaris 27.4 msec., tibialis anticus 24.3 msec., gastrocnemius 22.5 msec., and inferior oblique 18.7 msec.

TABLE 1

Average times to various peaks of mechanogram and electrogram of single twitch
All peaks were not present in all experiments

MUSCLE	RECORD	MSEC. TO PEAKS		
		#1	#2	#3
Diaphragm	Myogram	172 (10)	518 (10)	
	Electrogram	4.1 (2)	15.6 (2)	
Soleus	Myogram	35.8 (3)	103 (6)	
	Electrogram	2.3 (6)	4.6 (6)	6.5 (1)
Sartorius	Myogram	28.7 (7)	76.6 (5)	
	Electrogram	3.4 (6)	5.3 (3)	
Plantaris	Myogram	27.4 (2)		
	Electrogram	2.8 (2)	4.3 (2)	
Tibialis anticus	Myogram	24.3 (6)		
	Electrogram	2.3 (6)	4.2 (6)	7.0 (3)
Gastrocnemius	Myogram	22.5 (2)		
	Electrogram	2.1 (2)	4.6 (1)	
Inferior oblique	Myogram	18.7 (3)		
	Electrogram	1.3 (2)	1.8 (2)	

Parenthetical figures represent the numbers of animals giving the responses averaged to obtain the mean values given here.

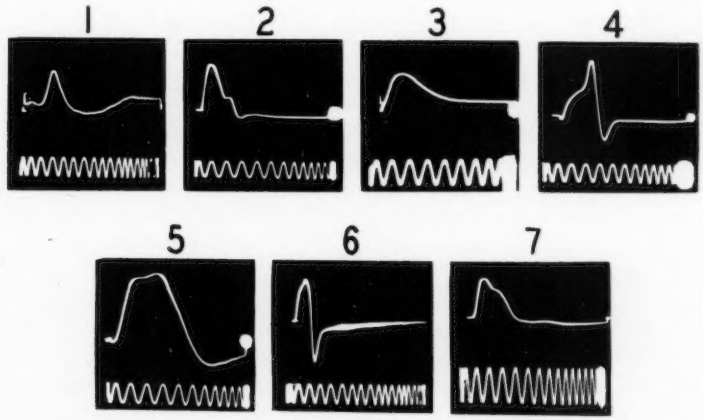

Fig. 2. Spread-out spikes of action potentials of muscles. 1—diaphragm, 2—gastrocnemius, 3—inferior oblique, 4—plantaris, 5—sartorius, 6—soleus and 7—tibialis anticus. Calibrations (in cycles per sec.): 1, 500; 2, 500; 3, 1000; 4, 500; 5, 500; 6, 200; 7, 500.

It will be noted that the three slowest muscles (diaphragm, soleus and sartorius) have two peaks of tension in the mechanogram. In no case, however, do either the fast or the slow components have the same properties in different muscles. This supports the previous conclusion (Rosenblueth, Wills and Hoagland, 1941) that while various muscles may be composed of a mixture of slow and fast fibers, these elements have different properties in different muscles.

The order of speed in reaching the principal peak in the electrical record was not the same as for the mechanogram. The mean times for development of maximal negativity were: sartorius 4.5 msec., diaphragm 4.1 msec., soleus 3.9 msec., plantaris 3.7 msec., tibialis anticus 3.4 msec., gastrocnemius 2.0 msec., and inferior oblique 1.6 msec. While the agreement between the orders of reaching the principal peaks in the mechanogram and electrogram respectively is not good in detail, there is a general tendency for the faster muscles to require less time for development of maximal negativity. Sartorius seems to be the principal atypical muscle in this regard.

TABLE 2

Average frequencies of stimulation for appearance of early "stages"

MUSCLE	NUMBER OF EXPERIMENTS	FREQUENCY FOR APPEARANCE OF:		
		Tetanus	Stage 2	Best sequence: 1, 2, 3a, 3b, 3c
Diaphragm	5	36	350	500
Soleus	4	22	225	340
Sartorius	4	23	275	465
Plantaris	5	25	280	430
Tibialis anticus	9	33	300	465
Gastrocnemius	7	31	250	400
Inferior oblique	6	69	700	1000

Table 2 gives the average values of the frequencies of stimulation of motor nerves necessary for the appearance in the corresponding muscles of tetanus, stage 2 and the clearest sequence of stages 1, 2, 3a, 3b and 3c, the muscles being arranged vertically in the table in order of increasing speed of contraction. The nomenclature of the "stages of transmission" used here is that of Rosenblueth and Cannon (1940).

It is evident that there was a general, but not very exact, parallelism between the speeds of contraction of the various muscles and the frequencies given in table 2. Diaphragm is the most striking exception to this generalization: while having a quite slow speed of contraction (table 1), it requires some of the higher frequencies of nerve stimulation for appearance of the various stages of contraction. The parallelism between the frequencies required for appearance of tetanus and of the other early stages is good, but not perfect.

A study of the late stages (4 and 5) has been made with respect to their relative tension productions in per cent of the tension of stage 1. The data are presented in the form of averages in table 3. This table contains also the mean

times elapsed from the beginning of nerve stimulation to the appearance of stage 5 for different frequencies of stimulation, and the frequencies of appearance of stage 5 in the various nerve-muscle systems.

It can be seen from table 3 that the 5th stage of neuromuscular transmission was obtained in all the systems studied in this work, but most consistently in soleus, plantaris and gastrocnemius. In sartorius, tibialis anticus and inferior oblique the 5th stage was obtained in about one half the experiments, while in

TABLE 3

The late "stages of neuromuscular transmission" in various muscles

MUSCLE	STIM. FREQ.	NO. OF EXPTS.	NUMBER OF 5TH STAGES	MINS. TO 5TH STAGE	4TH STAGE IN PER CENT OF 1ST	5TH STAGE IN PER CENT OF 1ST
Diaphragm	60	6	1	130	38.0	77.0
	120	1	0			
Soleus	60	3	3	63	7.1	40.9
	75	2	2	10	1.9	27.2
	120	1	1	4	10.3	66.6
Sartorius	60	4	3	67	3.2	19.5
	90	1	0			
Plantaris	60	3	3	71	9.6	26.4
	75	3	3	26	10.1	22.8
	90	1	1	30	11.0	22.0
	120	1	1	4	22.0	33.0
Tibialis anticus	60	7	3	53	1.2	7.7
	75	1	1	6	2.4	8.9
	85	2	1	10	0.4	1.4
	100	2	1	6	0.0	2.0
Gastrocnemius	60	6	6	83	6.7	23.9
	75	1	1	18	12.8	44.2
	120	1	1	10	7.2	31.1
Inferior Oblique	60	1	0			
	75	1	1	60	9.6	52.0
	120	1	0			
	150	1	1	10	15.3	18.4

diaphragm it appeared only once in 7 experiments. These results, in conjunction with those of table 2, allow us to conclude that the sequence of the several stages of transmission is quite general in nerve-muscle systems.

Maltesos and Weigman (1939) have found that stimulation of the chorda tympani at 425 cycles per sec. produced secretion by the submaxillary gland of a phasic nature, reminiscent of the sequence 1, 2, 3a, 3b and 3c in a nerve-muscle preparation. Wills (1941) has reported that chorda stimulation at frequencies

of 28.6 to 60.0 per sec. gave a series of changes of rate of secretion by the submaxillary gland analogous to the sequence of "stages" seen in a nerve-muscle preparation at relatively low frequencies (Rosenblueth and Morison, 1937; Rosenblueth and Luco, 1939). This evidence might suggest that phasic response to stimulation is a general property of nerve-effector systems, and not peculiar to nerve-muscle preparations. Lanari and Rosenblueth (1939), however, found no evidence of phasic changes of transmission in the nictitating membrane when the sympathetic postganglionic nerve supply was stimulated at various frequencies.

Table 3 shows that there was a tendency for the slower muscles to have, during stages 4 and 5, larger residues of the initial tension than the faster ones had. Gastrocnemius was the chief exception to this generalization, during stages 4 and 5 having greater tensions in per cent of that of stage 1 than might be expected from its position in the table. It is also apparent from table 3 that, in all cases, increasing the frequency of stimulation brought about earlier appearance of stage 5, as had been reported previously by Rosenblueth and Luco (1939).

SUMMARY

The number of peaks of negativity found in the monophasic action potential of muscle seems to have no definite relation to the number of peaks of tension appearing in the myogram (figs. 1 and 2; table 1).

While various muscles seem to be composed of a mixture of slow and fast elements, these elements in different muscles have different properties. Thus, there is no typical slow or fast fiber (table 1).

There is a general parallelism between the speed of contraction of a muscle and the frequency of motor nerve stimulation needed for the appearance of the various stages of neuromuscular transmission (table 2). In the late stages there is a tendency for the faster muscles to develop less tension in per cent of that during stage 1 than the slower muscles produced (table 3).

The sequence of stages of transmission seems to be general in striated nerve-muscle systems.

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ACCOMMODATION IN MAMMALIAN MOTOR NERVES

A. ROSENBLUETH AND E. C. DEL POZO[1]

From the Department of Physiology in the Harvard Medical School

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The term "accommodation" (Nernst, 1908) is used to describe a rise of the threshold of a nerve fiber when an electric current passes through it. This rise of threshold explains why the response of a nerve to a prolonged application of direct current may be a single impulse. A rise of threshold also explains why slowly increasing currents may fail to stimulate even though the final intensity reaches a value many times greater than the rheobase.

Following Hill's (1936) theoretical suggestions, Soldant (1936a, b) measured the time constant of accommodation of several nerves and found values of 42 to 83 msec. for mammalian motor nerves. These figures imply that progressively increasing currents which do not attain the value of 1 to several (n) rheobases within 1 to n times the time constant (about 60 msec.) will fail to stimulate. In other words, a current which increases progressively to an intensity 10 times greater than the threshold for suddenly applied direct current should reach that value within less than about 0.6 sec. in order to stimulate. A further implication is that if a constant current of intensity 10 times the rheobase is applied to a nerve, repetitive discharges should occur for less than 0.6 sec.

Rosenblueth (1940) frequently observed repetitive discharges for as long as 15 sec. or more when cats' motor nerves were treated with direct currents only 3 to 6 times stronger than the threshold. The discrepancy between these observations and Solandt's (1936a) measurements, interpreted on the basis of Hill's (1936) theory of accommodation, led to the present study.

METHOD. In cats, under dial anesthesia (Ciba, 0.7 cc. per kgm. intraperitoneally) a tracheal cannula was inserted. An Achilles tendon was dissected and tied, and the region of the calcaneum where it inserts was cut. The thread on the tendon was attached to a tension myograph pulling against rubber bands and recording the contractions of the muscles on a kymograph. The muscles were used as indicators of activity of the motor fibers in the popliteal nerve. The leg was fixed by means of drills inserted into the tibia.

The sciatic nerve was cut as high in the hip as possible. The hamstring nerves were cut and dissected away from the sciatic. The cut peripheral end of the nerve was tied and the trunk was dissected for about 2 cm., to the level of an arteriole and a venule which join the nerve about 0.5 cm. below the trochanter. These vessels were carefully preserved and, in addition, throughout the dissection the vessels of the nerve were respected. The importance of the circulation will be emphasized later. The segment of nerve dissected as above was sometimes found inadequately supplied with blood. The dissection was then con-

[1] Fellow of the John Simon Guggenheim Memorial Foundation.

tinued peripherally until a satisfactory stretch of nerve was found. Experience showed that consistent and stable results were obtained if, and only if, the two arterioles which run between the peroneal and the popliteal nerves were visibly carrying blood after the central end of the sciatic was tied and the stretch was freed that was to be introduced into the glass support of the electrodes.

The electrodes were placed as follows. The nerve was threaded into a glass tubing about 3.5 cm. long, supported by a glass rod held by a clamp. A sopper fixed the thread tied to the nerve and also prevented desiccation. The glass tubing had two short, open side-branches, with a distance of 1.5 cm. between them. Through these side-branches were introduced the wicks, soaked with Ringer, which connected the sciatic with the impolarizable electrodes. The wick distal to the muscle was in contact with the damaged region of the nerve between the cut and the tie. The other wick was in contact with normal tissue.

Two sets of electrodes were used, with similar results. Some were large calo-

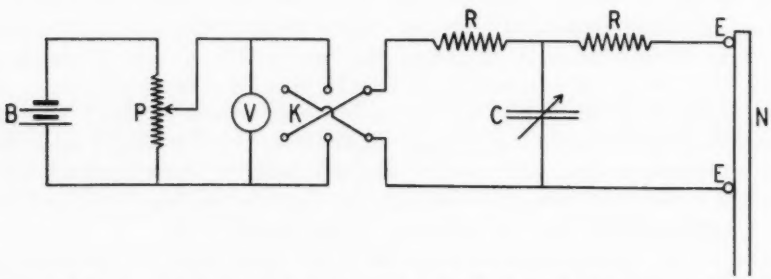

Fig. 1. Diagram of the stimulating circuit. *B:* 22.5 to 45 v. battery. *P:* 50,000 ω potentiometer. *V:* voltmeter. *K:* reversing key. *R:* 100,000 ω. *C:* variable capacity, 0 to 30 μ F. *E:* impolarizable Hg-HgCl or Ag-AgCl electrodes. *N:* nerve.

mel half-cells, the others large chlorided silver plates. The bridge from these electrodes to the nerve was first a short column of agar-Ringer, then the wicks wet with Ringer, mentioned above. The impolarizability of the electrodes within the range of currents employed in the experiments was tested by reading the current corresponding to different voltages from an ammeter in series with the electrodes and nerve. The system was found to obey Ohm's law accurately, thus showing that no counter e.m.f. of polarization developed at the electrodes with the highest voltages employed.

Exponentially increasing currents were obtained by the circuit in figure 1. The resistance of the electrodes, repeatedly measured, was 10,000 ω; that of the nerves, also repeatedly measured, varied from 3,000 to 5,000 ω. The total effective resistance of the circuit, through which the shunt capacity was charged, varied with the resistance of the nerve and with the different settings of the potentiometer. This variation was only slight, however, the extremes being 53,000 and 55,000 ω. No significant error was made, therefore, by using the average resistance of 54,000 ω in all instances for the calculation of the time con-

stant of the exponential rise of the currents applied. The time constant (in microseconds) was equal to the capacity (in microfarads) times 54,000.

The currents were sent through the nerve first in a descending direction (cathode towards the muscle), then in the opposite direction. The results are mainly concerned with the action of descending currents.

Hyperventilation was produced by connecting an artificial respiration pump with the tracheal cannula and by increasing the positive pressure of the pump to the desired degree, without changing the rate. Hypercapnia was obtained by delivering a moderate continuous stream of CO_2 through a narrow glass tubing inserted into the tracheal cannula. The animal breathed spontaneously in these cases. It promptly developed polypnea, but the consistent experimental results showed that a steady equilibrium was soon reached and that the CO_2 pressure in the arterial blood was increased.

RESULTS. A. *Accommodation in normal nerves.* The currents passing through the nerve in the circuit of figure 1 rise exponentially with a time constant determined by the capacity to a peak value proportional to the voltage indicated by the voltmeter. When currents of progressively slower rate of exponential rise are tested the peak voltage for threshold excitation increases gradually. This increase may be interpreted as due to the rise of threshold during the passage of the current. The curves correlating the time constant of the rising currents with the corresponding peak voltage necessary for stimulation may thus be used for the study of accommodation (see Hill, 1936). The experimental procedure was as follows.

The threshold for direct current, which is equivalent to an instantaneous exponential rise, or to a time constant 0, was first determined for a response of small amplitude. A pointer writing a horizontal line on the kymograph was adjusted to mark the amplitude of response selected. The variable capacity of figure 1 was then set to give different time constants for the currents and the corresponding threshold voltages were determined, maintaining the amplitude of response fixed. The time constants were first progressively increased, then decreased, and finally varied at random. In each observation, as mentioned under Method, a descending current was followed by an ascending one, but the measurements were made for the descending stimuli.

In some preparations a progressive decrease of excitability of the nerve was apparent, whether the tests were made with currents of a single or of different time constants. Experience showed that this progressive deterioration of the nerve was due to deficient blood supply. By shifting the electrodes, some region of the nerves was always found where the threshold for any given current did not vary for over an hour, the time necessary for the construction of a peak voltage-time constant curve. Only the observations made under this stable condition will be considered.

The peak voltage-time constant curves of normal nerves were of two different types. Figure 2A illustrates a characteristic example of the first type. The data from which this curve was constructed are given in table 1a. The voltmeter could be read with a limit of error of $\pm$ 0.03 v. A change of 0.05 v. in

the currents was readily measurable in the amplitude of the responses evoked. The accuracy of this and the following curves is therefore within about 2 per cent for the small time constants, and better than that for the higher points.

In contrast with the smooth curve of figure 2A the upper curve of figure 3A (table 1b) exhibits a break. This was typical of the second type of curves encountered. The break separated segments of curves corresponding to different processes, as indicated by differences in the responses to stimuli belonging to one or the other segment. Figures 4A and C illustrate these differences. All the responses caused by stimuli which plotted in the lower segment of the curves

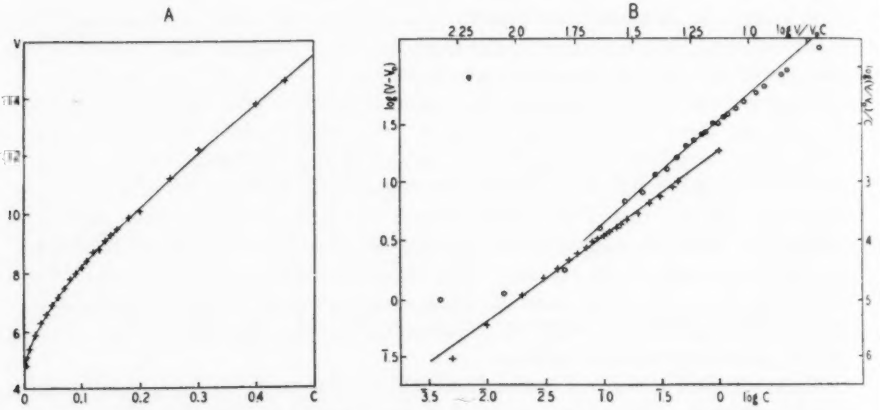

Fig. 2A. Normal peak voltage-time constant curve. Ordinates: voltage readings on voltmeter in figure 1; this value is lineally proportional to the peak value of current through the nerve, approached exponentially; the peak current may be obtained by dividing the voltage by 220,000. Abscissae: shunt capacity (fig. 1) in μF; the time constant (in msec.) of the exponential rise of current through the nerve may be obtained by multiplying the capacity (in μF) by 54. The curve indicates what peak voltage was necessary for threshold stimulation (constant small muscular response) at the corresponding time constant. The order in which the observations were made is listed in table 1a.

B. Crosses (lower scale of abscissae and left scale of ordinates): test of the points in A for a parabola (see p. 634). Circles (upper and right-hand scales): test for Hill's (1936) formula (see p. 639).

were brief, as in figure 4A; all those evoked by currents which plotted in the second segment were prolonged, as in figure 4C. At or near the point at which the break in the curves took place it was possible sometimes to observe double responses, a brief contraction followed by a sustained development of tension (fig. 4B).

The records in figure 5 illustrate the responses to suprathreshold currents of constant peak voltage and variable time constant of rise. It is noticeable that as the time constant increases the decrease of response is mainly at the expense of the early sharp peak elicited by instantaneously rising direct current (fig. 5, 1st response) rather than at the expense of the later relatively sustained tension.

These records may be interpreted on the assumption that the response to direct current involves an early component, which accommodates rapidly, and a later component, which accommodates more slowly. With the slowly rising currents the first component is absent.

The break in the curves of the second type was often quite obvious, as in figure 3A (crosses); it was sometimes not readily detectable (see fig. 8A, circles). The change in the type of response, brief or sustained, evoked by different currents,

TABLE 1

Data from which were constructed the curves in figures 2A (curve a), 3A crosses (curve b), 3A circles (curve c), 8A crosses (curve d), 8A circles (curve e), 9A crosses (curve f), and 9A circles (curve g)

The table shows the order in which the observations were made

CURVE A		CURVE B		CURVE C		CURVE D		CURVE E		CURVE F		CURVE G	
c	v	c	v	c	v	c	v	c	v	c	v	c	v
0	4.8	0	3.0	0	1.5	0	2.5	0	1.15	0	1.9	0	4.2
0.1	8.2	0.1	3.8	0.1	2.0	0.1	4.8	0.25	3.9	0.15	4.5	0.15	8.4
0.07	7.5	0.2	4.4	0.2	2.3	0.2	6.2	0.35	4.4	0.05	3.1	0.1	7.4
0.03	6.3	0.4	5.5	0.4	2.75	0.3	7.3	0.45	4.8	0.25	5.3	0.2	9.3
0.02	5.9	0.05	3.45	0.8	3.4	0.4	8.2	0.55	5.2	0.35	6.2	0.3	10.7
0.01	5.4	0	2.9	0.3	2.5	0.5	9.3	0.65	5.4	0.3	5.7	0.05	5.9
0.005	5.1	0.6	6.3	0.6	3.15	0.35	7.8	0.75	5.6	0.2	4.9	0.4	12.0
0	4.8	0.9	7.5	1.0	3.6	0.25	6.8	0.5	5.0	0.1	3.8	0.35	11.4
0.05	6.95	0.15	4.1	1.5	3.8	0.15	5.6	0.4	4.5	0.02	2.4	0.25	9.8
0.08	7.8	0.3	4.9	3.0	4.0	0.05	4.1	0.3	4.1	0	1.75	0.13	7.8
0.11	8.4	0.5	5.9	4.5	4.15	0.02	3.3	0.2	3.4	0.4	6.4	0.08	6.7
0.13	8.8	0.8	7.2	3.7	4.1	0.6	10.0	0.1	2.6	0.08	3.5	0.03	5.1
0.16	9.5	1.2	8.6	2.2	3.9	0.7	10.8	0.05	2.15	0	1.75	0	3.8
0.06	7.2	1.5	9.2	1.2	3.75	0.45	8.7	0	1.1				
0.04	6.6	0.7	6.75	0.5	3.0	0.55	9.6	0.15	3.1				
0.2	10.1	2.0	9.6			0.9	12.0	0.25	3.7				
0.25	11.2	3.0	10.2					0.9	5.7				
0.09	8.0	1.0	8.0					1.0	5.75				
0.3	12.2	0.25	4.7					0.02	1.65				
0.4	13.8	4.0	10.6										
0.14	9.1	0	3.2										
0.45	14.8												
0	4.9												
1.0	23.5												
0.18	9.9												

however, was always striking. That the curves had two segments whenever the two types of response were encountered was shown by a break in the linear test of the curves, which will be described later (p. 640).

The position of the break in the curves varied for different animals. It sometimes was present at short time constants (fig. 3A), while in other cases it did not become apparent unless quite long-time constants were tested (up to 0.15 sec.; 3μF). In nerves which yielded smooth curves similar to that in figure 2A,

if currents were applied with such long time constants, sustained responses were invariably obtained—i.e., the break in the curve was revealed. It may be inferred, therefore, that a break in the curves is a general phenomenon, not the occasional property of some nerves. In some cases, however, the second segment may only be seen with very slowly rising currents which require a correspondingly high voltage for stimulation. Such high voltages—i.e., over 20 v. (more than 0.1 mamp.)—may cause irreversible or very slowly reversible changes in nerve. The curves were carried out to points which did not require unduly high currents; for this reason some do not show the break.

In some experiments the resistances of 100,000 ω, usually employed, were changed to 250,000 ω, thus multiplying the effective resistance of the circuit by 2.5. Observations were made with capacities as large as 30μF. A time con-

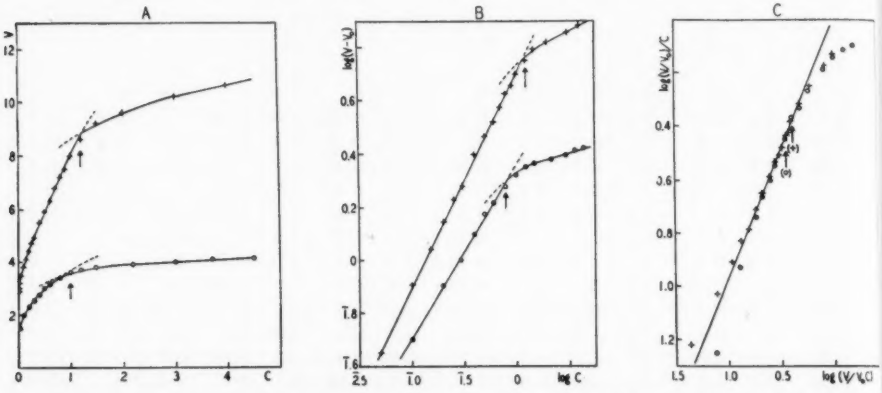

Fig. 3A. Peak voltage-time constant curves before (crosses, table 1b) and during (circles, table 1c) a prolonged period of hyperventilation. The arrows indicate the time constant at which the type of response recorded changed: to the left of the arrows the muscular responses were brief twitches; to the right, the responses were relatively sustained tetani.

B and C. Tests of the curves in A for a parabola and for Hill's formula, respectively.

stant of 4 sec. was thus tested. In all cases responses were obtained with peak voltages no greater than about 10 times the rheobase, i.e., less than 0.1 mamp. The responses could have latencies as long as 6 sec. and were all of the sustained type. Although, as mentioned above, intense currents were found to produce slowly reversible changes of excitability, the responses elicited by these slowly rising currents could be repeatedly obtained with only a slight progressive intensification of the peak voltage. Figure 4D illustrates a typical delayed and sustained contraction caused by a current reaching a peak intensity of 0.08 mamp., with a time constant of 3.9 sec.

B. *Hyperventilation.* Hyperventilation, with the consequent acapnia, promptly resulted in marked changes of electrical excitability and accommodation of nerve. In figure 6A is illustrated the change of rheobase. As soon as hyperventilation was started the rheobase decreased, as evidenced by the incre-

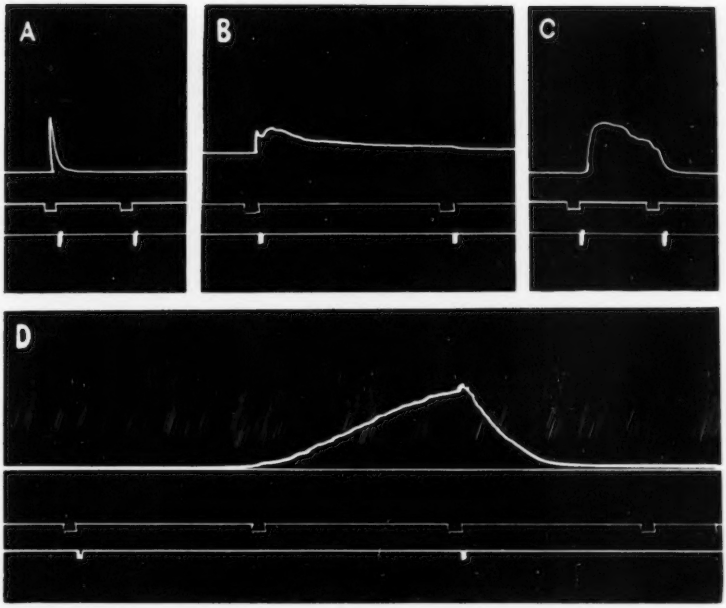

Fig. 4. Different types of muscular responses elicited by currents of progressively slower rise. Contractions of the Achilles tendon muscles. Time signal; for A and C, 2 sec.; for B and D, 5 sec.; the speed of the kymograph was the same for all records. The currents were applied between the lower signals.

A. Time constant of the exponential increase of the current: 27 msec. Asymptotic voltage indicated by the voltmeter in figure 1: 15 v.

B. Time constant: 43.2 msec. Voltage: 20.

C. Time constant: 162 msec. Voltage: 25.

D. In another animal. The 2 resistances R in the circuit of figure 1 were increased to 250,000 ω each. Time constant: 3,900 msec. Voltage: 40. Intensity: 0.08 mamp.

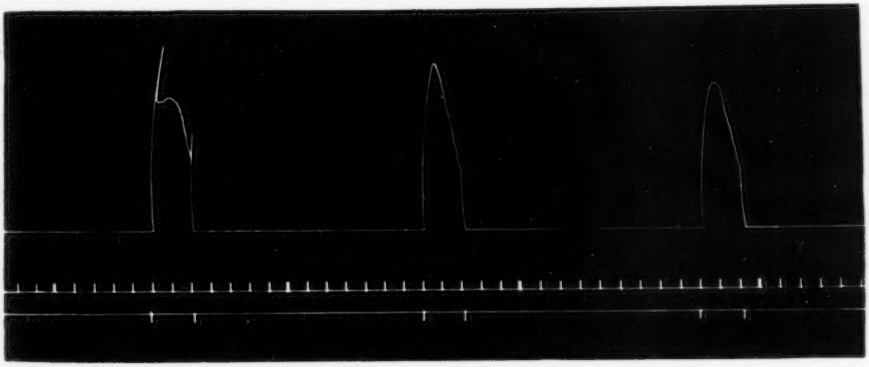

Fig. 5. Muscular responses to currents of constant peak voltage and various rates of exponential rise. The peak voltage was 4 times the rheobase. Time signal: 5 sec. Between the successive pairs of lower signals descending currents were applied with the following time constants for the rise: 0; 1.3; and 2.0 sec.

ment of the responses to direct-current descending pulses of constant intensity. The threshold to the make of the ascending currents was also lowered, as shown by the appearance of responses to that stimulus, absent before hyperventilation.

Not only did acapnia result in an increase of the rapidly accommodating, brief responses which direct currents may elicit, but also in an increase of the long sustained effects which may follow these brief responses (fig. 7). It may be concluded that the threshold for these prolonged responses is also lowered by hyperventilation.

As shown by figures 6A and 7, the changes produced by hyperventilation took place at first rapidly and then more gradually. After 10 to 15 min., how-

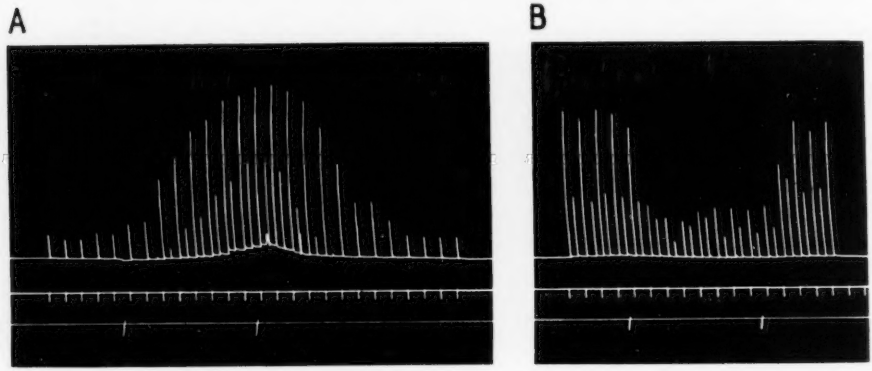

Fig. 6. Effects of hyperventilation and of hypercapnia on the rheobase. Time signal: 30 sec. Direct current pulses of 1 sec. duration were applied at 15-sec. intervals, with alternating polarity.

A. The intensity was the normal rheobase (1.2 v.). The lower signals indicate the beginning and end of a period of hyperventilation. The responses before hyperventilation correspond to the make of the descending currents. These contractions increase during hyperventilation, and in addition responses to the make of the ascending currents appear and increase progressively.

B. Intensity 5 times the normal rheobase. Stimuli applied as in A. Between the lower signals a moderate stream of CO_2 was delivered to the tracheal cannula. The large contractions before CO_2 correspond to the make of the descending currents and the small contractions to the make of the ascending currents. Both responses decreased during the period of hypercapnia, particularly the responses to descending currents.

ever, a steady equilibrium was reached, and it was then possible to make the series of observations necessary for the construction of a peak voltage-time constant curve. Figures 3 and 8 illustrate typical experiments. The whole curve during hyperventilation was lower than the normal curve—i.e., for any time constant of the exponentially rising currents less voltage was necessary for stimulation than in the normal nerve. The change of threshold was especially noticeable for slowly rising currents, those which plot as the second segment of the curves, after the break. The break occurred at a lower time constant than normal (fig. 3A); it sometimes became apparent only during hyperventilation (fig. 8A).

The degree of hyperventilation determined the magnitude of the changes produced. It was thus possible to cause relatively small changes by moderately

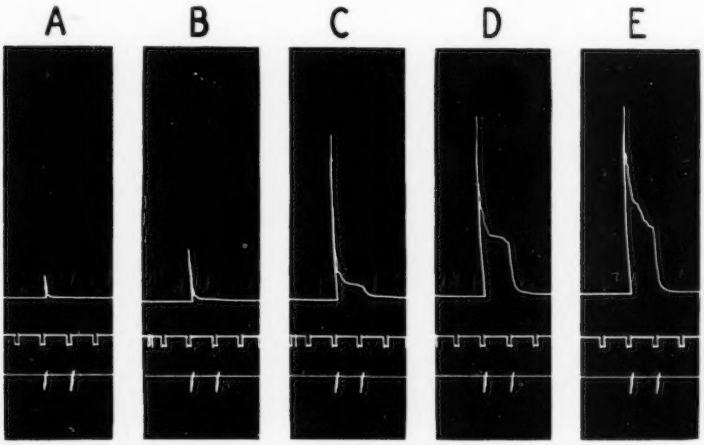

Fig. 7. Increase of the repetitive discharge of nerve in response to direct currents during the course of hyperventilation. Time signal: 2 sec. Descending direct current pulses of rheobasic intensity (1.2 v.) were applied for 2 sec. at 1-min. intervals.

A, normal control.

B to E, 1, 3, 5 and 7 min. after beginning of hyperventilation.

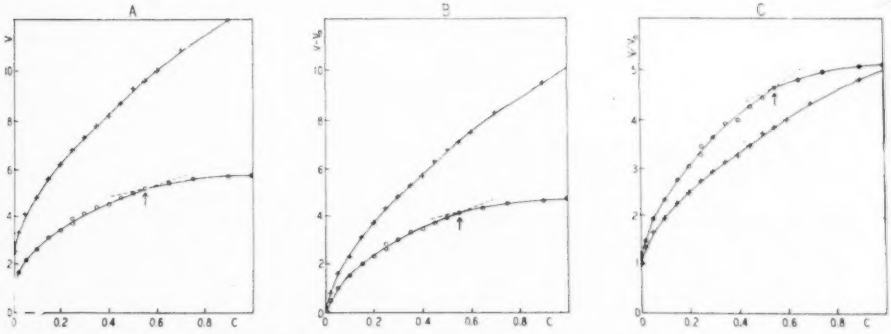

Fig. 8A. Peak voltage-time constant curves before (crosses, table 1d) and during (circles, table 1e) hyperventilation. The arrow has the same meaning as in figure 3.

B. As in A, but ordinates: difference between the peak voltage for a given time constant and the rheobase (time constant 0).

C. As in A, but ordinates: ratio of the peak voltage for a given time constant to the rheobase.

abundant artificial respiration; a later increase of ventilation resulted then in more marked effects.

As illustrated in figure 6A, the changes produced by hyperventilation were not permanent. Removal of the artificial respiration was followed by a return

toward the normal condition. With moderate and not very prolonged hyperventilation a satisfactory reversibility of effects was seen. This is the case in figure 6A. With strong and prolonged artificial respiration hysteresis became apparent. The nerve did not return to its normal characteristics but usually overshot—i.e., the rheobase and the peak voltage-time constant curve were higher than normal after recovery from a period of increased ventilation.

The sensitivity of the nerves to changes in respiratory rate and depth deserves emphasis. Even very slight hyperventilation resulted in striking changes similar to those illustrated in figures 3, 6A and 8. In some animals a sudden change of the spontaneous respiratory activity caused, for instance, by turning on a heating pad, could produce a significant shift of the peak voltage-time constant curve.

C. *Hypercapnia.* Increase of the CO_2 tension in the blood caused, like hyperventilation, prompt and marked changes in the nerves. As shown in figures

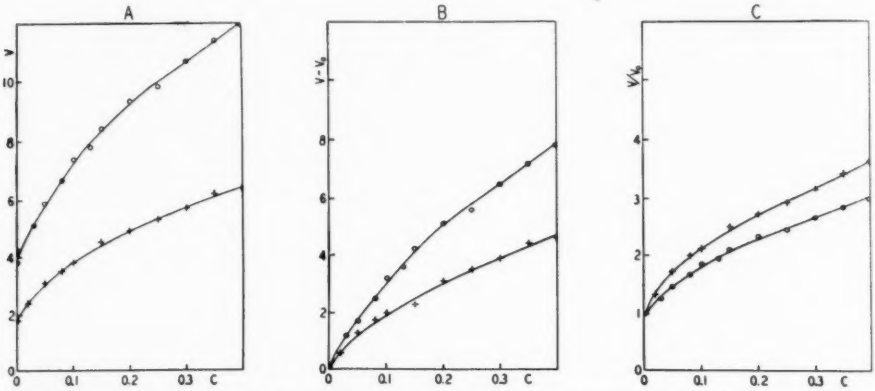

Fig. 9. As in figure 8, but illustrating the effects of hypercapnia. Normal curve: crosses (table 1f). During hypercapnia: circles (table 1g).

6B and 9A, these changes were opposite to those produced by acapnia. With increased CO_2 the rheobase rose; the peak voltage-time constant curves also rose, particularly the second segment of the curves.

As was the case with hyperventilation, the effects of increased CO_2 were totally (fig. 6B) or partially reversible. Hysteresis was seen here as an undershooting —i.e., as an incomplete return to normal—instead of the overshooting observed after recovery from hyperventilation.

D. *Veratrine.* The reasons for testing the effects of this drug will be stated in the discussion. The results were similar to those described above for hyperventilation (figs. 3 and 8); they are therefore not illustrated further. The reversibility of these effects was not tested, because the action of the drug is quite long-lasting (several hours) in mammalian nerves.

Discussion. I. *The peak voltage-time constant curves.* In this section will be considered only the smooth curves, such as that in figure 2A, and the lower

part of the broken curves, such as that in figure 3A (crosses). The significance of the break is discussed in the next section.

The curves indicate what maximal intensity is necessary in order to stimulate with a given time constant for the rise of an exponential current. This intensity is not necessarily the same as that at which stimulation occurs. As a relatively slowly rising current is passed through a nerve the threshold increases progressively. If at any time the excitatory process developed by the current reaches the threshold, stimulation will ensue. The current, however, may not have reached its peak value at that time. Indeed, it is likely (see Hill, 1936) that with currents of slow exponential rise the current is significantly below its maximal value at the moment of threshold stimulation.

Although the voltages plotted do not correspond directly to the change of threshold, the curves provide an indirect measurement of this change. Thus, if a nerve in certain experimental conditions accommodated more rapidly or to a greater extent than normally, the curve obtained in these conditions would rise more steeply and to a higher level than the normal curve, as the time constants of the currents increase. The detailed interpretation of a given curve, or the comparison of different curves with each other, depends, however, on the definition of accommodation adopted and on the assumptions made for the influence of the intensity of the current on the rate and degree of accommodation.

Hill (1936) assumed that the final degree of accommodation to a current of any fixed intensity is always a constant value above the degree of excitatory process developed—i.e., that after direct current has been applied for a long time the threshold of the nerve to a test shock would be normal at the cathode of the direct current. He further assumed that accommodation is proportional to the degree of excitatory process developed by the current. From these assumptions he derived the following mathematical expression for the peak current-time constant curves:

$$I_2/I_0 = (a/\lambda)^{\frac{a/\lambda}{a/\lambda-1}} \tag{1}$$

where I_2 is the peak intensity for any given time constant, I_0 is the rheobase, a is the time constant of the currents, and λ is the time constant of the process of accommodation. This equation is supposed to be applicable for values of a greater than $10k$; k is the time constant of excitation of the nerve.

The value of k for the motor fibers to the Achilles tendon muscles was determined by Rosenblueth and Dempsey (1939) and by Rosenblueth (1940), using Hill's (1936) method, and was found to vary from 0.05 to 1.5 msec. Equation (1) should therefore apply to the present curves from the point corresponding to $0.2\mu F$ onwards. This applicability was tested lineally as follows. Equation (1) may be rewritten

$$\log (I_2/I_0) - \log (a/\lambda) = \lambda \log (I_2/I_0)/a \tag{2}$$

Since I_2 is proportional to the peak voltages, and a is proportional to the capacity, it follows from equation (2) that if $\log (V/V_0) - \log C$ is plotted against $\log$

$(V/V_0)/C$ (where V is the peak voltage, V_0 is the rheobase, and C is the capacity) a straight line should ensue.

Although occasionally a good lineal test developed, the results illustrated in figure 2B (circles) were the rule. Not only did the points deviate from a straight line for the small capacities, to which equation (1) is not supposed to apply, but the points corresponding to relatively high capacities also deviated from a straight line. It may be inferred that Hill's equation does not fit the experimental data, and hence that the assumptions which led to that formula are in turn contrary to experimental facts.

The following equation was found to fit with accuracy all the curves constructed during the experiments:

$$V - V_0 = m\, a^n \tag{3}$$

where V, V_0 and a have the same meaning as before, m is a proportionality factor and n is a fraction ($1 \geq n > 0$). According to this equation V–V_0, which is indicative of the rise of threshold, is a parabolic function of the time constant of the exponential rise of the currents. Equation (3) may be rewritten

$$\log (V-V_0) = \log m + n \log a \tag{4}$$

Accordingly, if equation (3) is adequate, the plot of log $(V-V_0)$ against log C should yield a straight line with slope equal to n. This lineal test is illustrated in figure 2B (crosses). As is the case in this figure, the points corresponding to very small capacities were frequently off the straight line, but their deviation was unsystematic. This deviation is attributed to the difficulty of measuring accurately both V_0 and the V corresponding to these points. The test is such that slight errors become greatly magnified at that range.

With the exception of the points corresponding to capacities less than $0.05\mu F$ (time constant less than 2.7 msec.) all the smooth curves yielded excellent lineal tests for a parabola. The first segment of the broken curves also gave a good lineal test (fig. 3B). The curves are therefore fully described if the 3 parameters V_0, m and n are determined. V_0 is measured experimentally. The other parameters can be directly read from the position of the straight line in the double logarithmic plot; n is the slope of the line and m is obtained from the intercept with the V–V_0 axis.

Hill's equation (1) reaches a limiting slope, hence Hill's suggestion that λ, the time constant of accommodation, may be calculated from this slope. The derivative of V-V_0 with respect to a in equation (3) is

$$d(V\text{-}V_0)/da = mn/a^{1-n} \tag{5}$$

This derivative is the slope of the curve and decreases indefinitely as a increases without limit. In other words, the parabola of equation (3) has no limiting slope. Since all the curves tested fitted equation (3) better than equation (1) it may be inferred that the use of a limiting slope to measure the time constant

of accommodation is not justified by the data. The second derivative of (3) with respect to a is

$$d^2(V\text{-}V_0)/da^2 = mn(n\text{–}1)/a^{2-n} \quad (6)$$

which indicates that the slope varies at first rapidly with a and then progressively more slowly as a increases. For this reason the curves may suggest a limiting slope, unless analyzed closely.

The average measurements for normal nerves were as follows. The rheobase was usually 3v. potential drop in the voltmeter V of figure 1, with extreme variations of 5.5 and 1.2v. These values correspond to an average current of 14μamp. (25 to 5.5), and to an average potential drop in the nerve of 55 mv. (100 to 22). The constant λ, measured according to Hill's (1936) method was 18 msec. (40 to 11.5). The constant n (equation 3) was 0.67 (0.83 to 0.48). There was no correlation between any of the three measurements—i.e., a low rheobase did not correspond consistently to any extreme value of λ or n, and similarly for the others.

Peak voltage-time constant curves for exponentially rising currents have been studied previously (see Cardot and Laugier, 1913; Schriever, 1930; Delville, 1934; Solandt, 1936a and b; Bernhard, Granit and Skoglund, 1942). In many cases the curves are similar to those illustrated in figure 2A and in the first segment of the curves in figure 3A. All the authors agree that the curves approach straight lines for currents of relatively long time constants. This interpretation is obviously a satisfactory first approximation (see figs. 2A and 3A). The analysis of the curve as a whole, however, shows that a parabolic equation with a fractional exponent gives a better description (figs. 2B, crosses, and 3B).

Curves which were straight lines from the start (including short time constants) were often seen by Cardot and Laugier, Schriever, and Solandt. Equation (3) becomes of course a straight line if n is equal to 1. In the present observations $n = 1$ was seen only in one nerve, after prolonged experimental handling—i.e., after several curves had been constructed in which n was less than 1. Invariably n was a fraction in fresh nerves. Usually, if an experiment was prolonged, after several procedures had been applied to the nerve, both the rheobase and n tended to increase. The straight lines ($n = 1$) observed by Cardot and Laugier, Schriever, and Solandt, were probably common in their experience, therefore, because these authors used excised nerves, as opposed to the circulated nerves observed here.

Curves with an initial concavity upwards, such as were reported by Delville, or curves which become a horizontal straight line for large time constants, such as were found by Bernhard, Granit and Skoglund, were never seen in our experiments.

II. *The significance of the break in the peak voltage-time constant curves.* Although, as pointed out above, the curves do not have a fixed slope, their slope at a given point denotes the rate at which the threshold changes when the current is made to rise more slowly—i.e., the slope is an indication of the rate of

accommodation. The break (see fig. 3A) suggests, therefore, a transition from a rapidly accommodating (steeper segment of the curve) to a slowly accommodating process (flatter segment of the curve). The type of response corresponding to each of the two segments agrees with this interpretation. The responses of the first segment are brief (fig. 4A), thus showing rapid accommodation, while those of the second segment are prolonged (fig. 4C), thus indicating slow accommodation.

It may be inferred, therefore, that the nerves have two time constants of accommodation. This inference leads to the further conclusion that the nerve has two thresholds to electric currents. These two thresholds may vary independently. Thus, hyperventilation (figs. 3 and 8), CO_2 (p. 638), and veratrine (p. 638), all affect the second segment of the peak voltage-time constant curves more than they affect the first segment—i.e., the slowly accommodating threshold is altered more than is the rapidly accommodating threshold. Similarly, in figure 7C to E, the slowly accommodating threshold is seen to decrease at a time during hyperventilation when the rapidly accommodating threshold is changing only slightly.

The existence of two independent thresholds leads in turn to the conclusion that the currents may stimulate nerve by two different independent processes. For rapidly rising currents the threshold of the rapidly accommodating process is lower. This type of response has been more frequently studied and some of the laws which preside in this mode of stimulation are therefore well understood. If a rapidly rising current is delivered which is higher than the two thresholds, a first rapidly accommodating burst of impulses may be followed by long sustained discharges (fig. 5, 1st response). With slowly rising currents, on the other hand, the threshold for the rapid mode of stimulation recedes ahead of the current, while the more slowly accommodating second threshold may be attained and a pure second mode of stimulation may ensue (fig. 4C and D; fig. 5, 3rd response).

III. *The measurement of accommodation. Hypo- and hypercapnia.* As shown in figures 3A, 8A and 9A the peak voltage-time constant curves of nerve are markedly modified by these experimental procedures. The question arises how to qualify these changes with regard to accommodation. Specifically the questions may be asked whether a nerve accommodates more or less than normally during hypo- or during hypercapnia. If the rheobase did not change, those questions would be easy to answer; a direct comparison of the curves would suffice. But since the rheobase is changed the answer is not obvious.

Accommodation has been defined as the increase of threshold during the passage of current. The degree of accommodation will then depend on the degree of change of threshold. Two possibilities are available for the comparison of different curves. The change in threshold may be measured as the difference between the currents necessary to stimulate after and before accommodation. In figures 8B and 9B are plotted these differences (V-V_0) against the corresponding time constant (C). The inference from the curves in these figures would be that nerves accommodate less than normally during hyperventilation, and more than normally during hypercapnia.

An alternative measure of the change in threshold is the ratio of the threshold after accommodation to the rheobase (V/V_0). In figures 8C and 9C are plotted such ratios against the time constant of the currents. The position of the curves in these figures suggests that nerves accommodate more than normally during hyperventilation, and less than normally during hypercapnia—i.e., a conclusion precisely opposite to that reached when the difference $V\text{-}V_0$, instead of the ratio V/V_0, is used as a criterion of accommodation.

To select either of the two criteria, $V\text{-}V_0$ or V/V_0, as the standard, is to define arbitrarily accommodation in one of the two possible ways. There is no obvious reason for preferring one or the other formulation. The main difference in the two procedures is that when $V\text{-}V_0$ is used the unit of threshold is the volt, or the ampere if the e.m.f. be converted into current, whereas when V/V_0 is employed the unit of threshold is the rheobase. The use of $V\text{-}V_0$ appears, therefore, preferable, because it adopts a standard unit and does not lay undue emphasis on the rheobase—i.e., it does not make the unnecessary assumption that the amplitude of the rheobase is a factor which determines the degree of accommodation.

This discussion of the measurement of accommodation does not follow the lines suggested by Hill (1936). As already mentioned, Hill assumed that after a sufficient time of application of a current of any intensity the threshold of the nerve would always be the same. This assumption is contrary to experimental fact. If the excitability at the cathode of direct current is measured at different times after the application of the current it is found that a level is reached after a brief period. This level is steady for minutes (up to 10 min.) and denotes an increase of excitability above normal, which is approximately proportional to the intensity of the current, within a wide range (unpublished observations).

Since Hill assumed an invariant degree of final accommodation the only measurement he considered of importance was that of the rate of development of accommodation. The data indicate, however, that the degree of accommodation is not constant, but is a function of the intensity of current. For this reason the discussion here has been concerned not only with the time constant of the rate of accommodation, but also with the degree of accommodation to a current of a given final intensity.

Summarizing the effects of hyperventilation on nerve, the following statements may be made. The most obvious change is in the rheobase, which can decrease to one-third the normal value. The constant λ, calculated by Hill's (1936) method, is practically unchanged. The value of n in equation (3) decreases. A comparison of the changes of threshold produced by slowly rising currents during hyperventilation with the normal changes indicates that hyperventilated nerves accommodate less than normally, if these changes are measured as a difference in the stimulating voltage (fig. 8B). During recovery from hyperventilation the changes were opposite in sign (p. 637). The effects of hypercapnia were also opposite in sign to those seen during hyperventilation (fig. 9).

IV. *Veratrine.* Among other striking effects of veratrine on nerve, characteristically the responses to single shock stimulation become repetitive (Dun and Feng, 1940; Acheson and Rosenblueth, 1941). These repetitive discharges occur long after the stimulus has ceased. They may, therefore, be quite un-

related to the phenomenon of accommodation, in which the interesting feature is the behavior of nerve during the passage of current, not afterwards.

This view differs from that adopted by Cowan and Walter (1937), who state that since nerves treated with tetra-ethyl-ammonium iodide respond repetitively to single shock stimulation, it was to be expected that their accommodation would be slower than normal.

The observations with veratrine emphasize that repetitive responses to single shocks need not be associated with a significant decrease of the rate of accommodation. The main change produced by the drug in the first segment of the peak voltage-time constant curves was to decrease the rheobase (p. 638). The slope of the curves at any given relatively large time constant, on the other hand, was only slightly decreased. The measurements of λ in a typical observation were 12.3 msec. before, and 11.3 msec. after a dose of veratrine sufficient to cause marked repetition.

Apart from the change of rheobase, the part of the curves most affected by veratrine was the second segment (p. 638). Since the change was similar to that produced by hyperventilation (figs. 3A and 8A), and since single shocks did not lead to repetitive responses during hyperventilation, it may be concluded that the repetitive discharges of veratrinized nerves do not depend on the effects of the drug on the second process of excitation and accommodation revealed by the second segment of the curves.

V. *Some properties of nerve suggested by the data.* In addition to the inference that currents may stimulate nerve by two different independent processes (p. 642), the data have a bearing on some aspects of other properties of nerve, as follows.

It has been classical to consider nerves as very resistant to the absence of circulation. Yet it is interesting to note that usually in studies on excised nerves some time is allowed to pass after dissection before the beginning of the study. The statement is frequently made (see e.g., Lucas, 1907) that during approximately the first hour after excision nerves are in an unsteady condition. Lucas (*loc. cit.*) considered two possibilities to explain this instability: the injury done by section and excision might be the responsible damaging factor; or else the nerve may slowly adjust from its normal to a new relatively steady state. The present data emphasize the importance of the circulation of nerve for optimum function. Section of the nerve did not cause instability, but inadequate circulation resulted in a progressive deterioration (p. 631).

The extreme sensitivity of some nerves to electric currents may be stressed. Normal nerve could have a rheobase as low as 1.2 v., measured at the voltmeter V in figure 1. During hyperventilation the threshold for direct current could drop to 0.8 v. In terms of current these values indicate thresholds of 5.5 and 3.8 μ amp., respectively. The drop of potential across the nerves was in turn only 22 to 15 mv., values which are of the order of magnitude of the usual demarcation potential.

The parabolic relationship (equation 3) found between the increment of threshold and the time constant of the exponentially rising currents entails an unexpected corollary. If the curves should remain parabolic beyond the range

explored, any current, no matter how slow its rise, would be capable of stimulating if the intensity were increased sufficiently. This corollary is in contradiction with the generally accepted notion (Lucas, 1907) that there is a limiting rate of rise of lineally increasing currents below which no stimulation will ensue no matter how strong the current becomes.

Admittedly the inference reached here, that a slowly rising current would always stimulate if it were made sufficiently intense, is an extrapolation of the curves in figures 2A, 3A, 8A and 9A. Practically a very slowly increasing current would destroy the nerve before stimulation. It should be noted, however, that Lucas' conclusion of a limiting slope of lineal increase is also an extrapolation to infinite. What Lucas observed experimentally was that currents with a slow gradient did not stimulate even when they ultimately reached a voltage 8 times the rheobase. In the present observations it was always possible to stimulate with currents of peak voltage of 6 to 10 rheobases, even when their time constant of rise was 4 sec., i.e., when after 4 sec. they had only reached an intensity of approximately 4 to 7 rheobases (fig. 4D).

SUMMARY

Accommodation, that is, a change of threshold during the passage of current, was studied in circulated cat's nerves by measuring the peak voltage for threshold stimulation by an exponentially rising current of variable time constant (fig. 1).

The peak voltage-time constant curves were smooth (fig. 2) or they had a break (fig. 3), thus indicating the existence of two segments. The responses plotted in the first segment differed from those plotted in the second (fig. 4). Both types of response, brief and protracted, could be seen with suprathreshold voltages and rapidly rising currents (fig. 5).

Hyperventilation and veratrine caused a decrease of rheobase (fig. 6A) and a lowering of the curves (figs. 3 and 8, p. 638); hypercapnia resulted in a rise of the curves (fig. 9).

The curves do not fit the theoretical equation which was suggested by Hill (1936; equations 1 and 2; figs. 2B and 3C). They are adequately described by a parabolic relationship between the increment of voltage and the corresponding time constant (equations 3 and 4; figs. 2B and 3B). This relationship implies that there is no limiting slope (equation 5).

The break in the curves denotes two different thresholds with different time constants of accommodation (p. 642). From these two thresholds it is inferred that currents may stimulate nerve by two different independent processes (p. 642).

The measurement of accommodation is discussed (p. 642). The existence of a critical lineal gradient for stimulation by direct current is rendered unlikely by the data (p. 644).

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SOME ACTIONS OF INDOLE ON THE DOG

HAROLD N. ETS AND I. M. FEINBERG
From Loyola University School of Medicine, Chicago

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Ever since the work of Herter (1), the effects of indole in the body have been discussed. The origin of indole is usually considered as intestinal, but Doland (2) mentions that morbid conditions of the mouth and throat as well as gangrene and abscess of the lung favor the production of indican. Some investigations have been made on indole in cold-blooded animals (3, 4), and also on isolated tissue of warm-blooded animals (5), but it is the purpose of the present paper to study the effect of indole on anesthetized dogs.

METHODS. Dogs were anesthetized with nembutal and prepared for recording arterial blood pressure, respiration and intestinal movements. The blood pressure was obtained from the carotid artery, respiration by means of a pneumograph about the thorax, and the intestinal movements by means of a balloon in the duodenum. The indole was dissolved in propylene glycol or in olive oil, all other drugs were dissolved in water. Injections were made into the femoral vein unless otherwise stated.

RESULTS. Twenty-two dogs were used. Figures 1A and 1B illustrate the typical effects obtained. Doses of 25 mgm. of indole per kilo produced rapid effects which gradually diminished after about 15 minutes. There is a momentary apnea followed by a marked increase in the rate and usually the depth of respiration. The blood pressure falls abruptly and then slowly and gradually returns to normal. Intestinal tone and movements varied: usually there was a fall of tone and movement, occasionally there was a rise in the tone with increased movements, and in a few cases there were mixed effects. Salivation was usually increased and mucous secretions accumulated about the nostrils. Spasms of the paws and jaws usually developed and frequently generalized clonic convulsions appeared, gradually disappearing after about 10 to 15 minutes.

Various methods of administration were tried and it was found that the usual doses given subcutaneously, intermuscularly, or orally were without effect. Intraperitoneal injections produced the same reaction as the intravenous injections.

Atropine in doses of 1 mgm. per kilo had no effect on the indole reactions on the blood pressure, respiration, intestines and convulsions, but it did seem to decrease salivary and mucous secretions.

Doses of 60 mgm. of indole per kilo injected intravenously caused intensification of all the above symptoms without a return towards normal. Death resulted from the continuous fall of blood pressure to zero. The autopsy of animals killed with indole showed the following: the lungs were edematous and

bloody mucous secretions were seen in the trachea, the abdominal organs showed a marked passive congestion and the heart was usually dilated.

Discussion. Yanai (4) has reported that indole will produce convulsions when injected into the frog. In the present work indole regularly produced convulsions when given intravenously or intraperitoneally.

Ott and Ulman (3) and Danilewski (6) report that indole lowers the force and the frequency of the frog's heart. Waddell (5) who studied the action of indole on excised warm and cold-blooded animals' hearts also noted a diminished ampli-

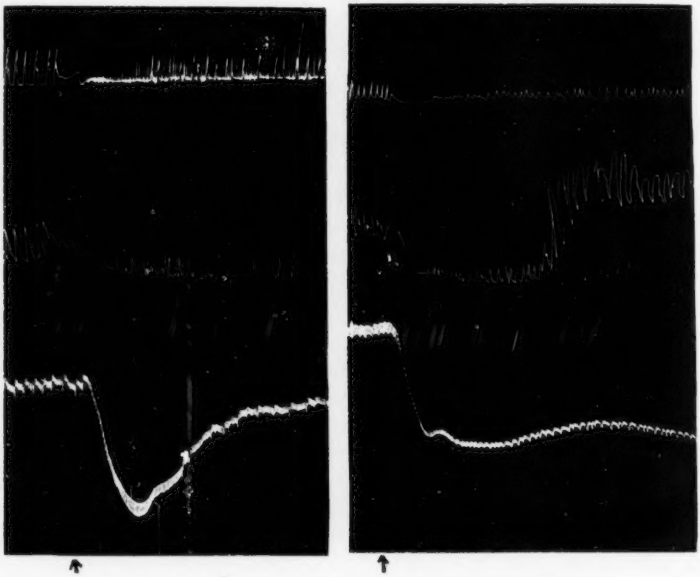

Fig. 1A Fig. 1B

Fig. 1. Upper tracing, respiratory record; middle tracing, intestinal movements; bottom tracing, blood pressure record. Figure 1A, 25 mgm. of indole per kilo of body weight were injected at arrow; note apnoea and convulsive movements with fall of intestinal tone and blood pressure. Figure 1B, 60 mgm. of indole per kilo of body weight were injected at arrow; note apnea, rise of intestinal tone, and fall of blood pressure.

tude and output. Biebl (7) reports a congestion of the liver and other organs from the action of indole. The above reports may explain the fall of blood pressure that was always observed in this work.

The salivation and mucous secretions reported here on the dog may be inferred from the work of Biebl who found that chronic effects of indole produced a pneumonia of the broncho-lobar type. Guggenheim and Loeffler (8) studied the action of indole on intestinal strips of guinea pigs and found that there was first a rise and then a fall of the tone. This also agrees with many of our experiments.

Waddell *loc. cit.* from his work believes that indole acts so as to depress the

heart muscle directly since atropine and indole seem to depress smooth muscle in general. We also found that atropine did not interfere with many of the actions of indole.

The duration of the effects is probably due to the fact that indole remains in the blood stream only a short time, as has been reported by Houssay.

SUMMARY

In dogs anesthetized with nembutal, indole injected intravenously in doses of 25 mgm. per kilo produces:

1. A short period of apnoea followed by an increase in respiration.
2. An immediate fall of systemic blood pressure.
3. Usually a rise in the intestinal tone followed by a fall.
4. Increase of mucous and salivary secretions.
5. Chronic convulsive movements (which with larger doses become generalized).
6. The effects are not abolished by atropine.

Death which occurs with large doses of indole is usually due to dilatation and arrest of the heart action.

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THE ETIOLOGY OF HYPERICISM, A PHOTOSENSITIVITY PRODUCED BY ST. JOHNSWORT

NELLO PACE

From The Division of Physiology, University of California, Berkeley[1]

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Light sensitivity has latterly become regarded as a discrete clinical entity. However, experimental studies of this general syndrome are rather scarce. In his recent monograph Blum (1) has presented an extensive and critical review of the entire field, and one of the most clear-cut photosensitivities he discusses is the disease of domestic animals known as hypericism. The disease has long been recognized as due to the grazing of domestic animals on the common weed St. Johnswort, *Hypericum perforatum*, with subsequent exposure to sunlight. Blum (1) points out the lack of certain evidence to establish the etiology of the disease. An inquiry into this problem seemed to promise not only a better understanding of hypericism, but a contribution to the general knowledge of photosensitization.

Hypericin, a red fluorescent pigment occurring in St. Johnswort, has been suspected as the causal agent owing to its activity as a photodynamic sensitizer. The pigment was recently studied chemically by Pace and Mackinney (2) and found to consist of several closely related chemical fractions. These fractions are believed to be partially hydrogenated polyhydroxy derivatives of helianthrone, which is related to anthraquinone.

The present study is an attempt to establish more exactly the relation of hypericin to the disease, and several lines of investigation have been pursued. First, the presence of hypericin in the skin of laboratory animals previously fed the dried plant was ascertained, and second, the ability of light of various wave lengths to produce characteristic symptoms of hypericism was studied in order to determine the approximate action spectrum. The latter should correspond to the spectral regions of maximal light absorption by hypericin if this pigment is the causal agent (1). In addition to these experiments the effect of oral feeding of purified hypericin was studied.

EXPERIMENTAL. *Demonstration of hypericin in the skin.* White rabbits were sensitized by feeding on dried *Hypericum perforatum* with no other food for at least one week. The sensitivity to light was tested by irradiating a small spot one square centimeter in area on the previously depilated flank of the animal by means of the apparatus shown schematically in figure 1. Five to ten minutes' irradiation in this apparatus with only filter F_2 in the optical path was sufficient to evoke a response in sensitized animals. The response, which took the form of a wheal surrounded by erythema and accompanied by symptoms of localized pruritis, was best observed on the day following irradiation. At this time an

[1] Present address: National Naval Medical Center, Bethesda, Md.

estimate of the degree of response could be made. After several days, a necrosed area occupying the site of irradiation was noted.

Three rabbits whose photosensitivity was tested in this fashion were killed and their skins, livers, and a portion of the skeletal muscle were ground up separately and extracted with 90 per cent acetone in water. These extracts were filtered and examined spectroscopically. The absorption spectrum of hypericin was readily recognizable in all three extracts. Hypericin in this

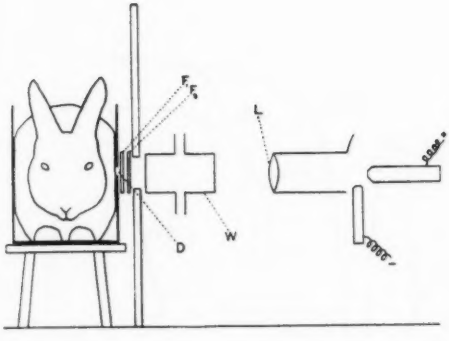

Fig. 1

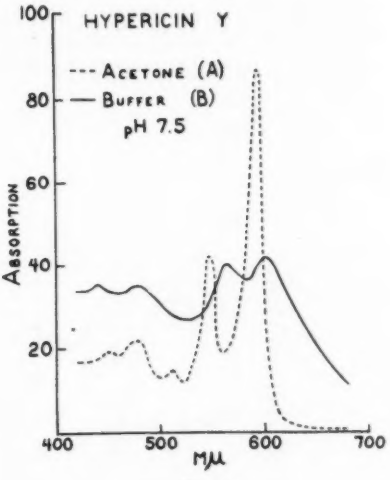

Fig. 2

Fig. 1. Apparatus for determination of action spectrum. The light source is a Leitz-Wetzlar D.C. projection arc using Eveready "Sunshine" carbons. A potential of 50 volts was maintained across the arc. The light was focused on the bare skin of the rabbit by means of the lens, *L*, and the diaphragm, *D*. The circular opening in the diaphragm 1 cm. in diameter served to sharply delimit the area irradiated. Permanently interposed in the optical pathway were two filters, *W* and F_1. *W* is a water filter 8.5 cm. in length which removed most of the infra-red. F_1 represents a Wratten gelatin filter (2-A) which is designed to cut off radiation of wave lengths below 400 mμ. The third filter, F_2, represents the series of Wratten gelatin filters used to isolate various portions of the spectrum.

Fig. 2. The ordinate is in relative units and does not indicate extinction coefficients of either solution. The figure is intended to show the qualitative difference between the two spectra.

solvent (curve A in fig. 2) displays an intense absorption band at 595 mμ with a weaker band at 550 mμ. Both bands could be detected in the extracts.

When reflected light from the skin of living sensitized rabbits was examined with a small direct-vision spectroscope, a faint absorption band could be discerned in the vicinity of 605 mμ. This corresponds approximately with the main absorption band of hypericin either in acetone or in aqueous solution buffered at pH 7.5. The absorption spectrum of hypericin in this medium is shown by curve B in figure 2. In addition to the faint band at 605 mμ the strong oxyhemo-

globin bands at 540 mμ and 577mμ were observed. Control animals examined in the same way did not show the additional band at 605 mμ nor did aqueous acetone extracts of normal tissues exhibit absorption bands at 595 mμ and 550 mμ.

These observations seem to establish definitely the presence of hypericin in the skin of animals fed on St. Johnswort and shown to be photosensitive.

Action spectrum of hypericism. The ideal manner for study of the action of various wave lengths of light on a photobiological process entails the use of monochromatic radiation to produce the response. However, since the intensities of light required to evoke a response in photosensitive animals are relatively high, this method is impractical. Consequently in the present work recourse

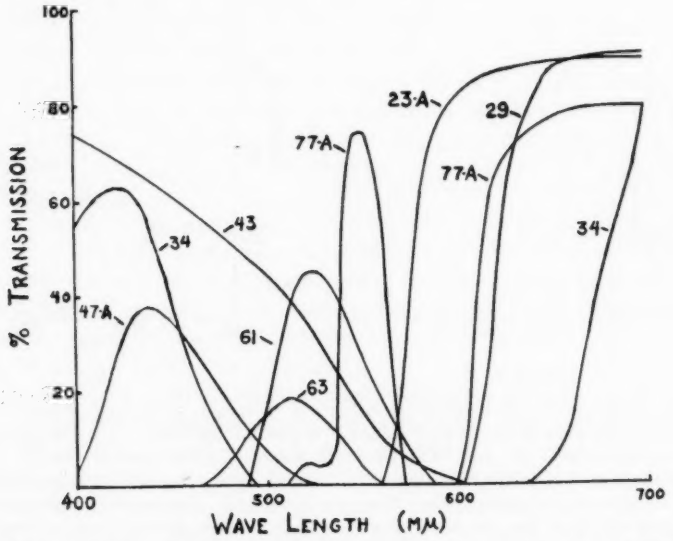

Fig. 3. Curves showing the per cent of light transmitted at various wave lengths by the filters used in this work. The numbers are the Wratten designation for the filters shown.

was had to the use of filters which strike a balance between maximal spectral purity and maximal energy transmission. A series of Wratten gelatin filters was chosen to cover the region of the spectrum from 400 mμ to 700 mμ. The per cent transmission curves for these filters are shown in figure 3.

Rabbits which had been previously sensitized by feeding on St. Johnswort were irradiated in the apparatus shown in figure 1. This was so arranged that the various filters in figure 3 could be interchanged at position F_2 in the apparatus. Tests were made with each filter on several rabbits. An estimate of the degree of response was made subjectively as described in the foregoing section, and results of the tests are given in table 1.

It was impractical to make more than three determinations on one rabbit because of the long exposures required. For this reason the first exposure was

always made through Wratten filter 23-A to check the animal's sensitivity since the response through this filter after twenty minutes of irradiation was indistinguishable from the maximal response with no filter in the optical path. Thirty minutes of irradiation was the standard period of exposure in all experiments. This period of exposure had no effect whatever on normal rabbits.

TABLE 1

Results obtained by irradiation of sensitized rabbits through various filters

The degree of erythema is indicated as follows: +++ strong; ++ medium; + barely perceptible; and 0 no effect. See table 2 for the wave-lengths transmitted by these filters.

RABBIT NO.	WRATTEN GELATIN FILTERS							
	23-A	29	34	43	47-A	61	63	77-A
1	++							
2	+++	0						
3	++		0					+
4	+++						0	++
5	+	0			0			
6	+++				0	0		
7	+++	0		++				
8	+++							++
9	+++		0				0	
10	++		0	+				
11	+++			++		0		
12	+++							

TABLE 2

Column 2 summarizes the results from table 1 for each filter used. Column 3 indicates the predicted ability of each filter to produce a response if hypericin is the photosensitizer. The limits of transmission of each filter are given in column 4. For further explanation see text

(1) FILTER	(2) AVERAGE RESPONSE	(3) AREA UNDER CURVE	(4) REMARKS
23-A	+++	13.44	Transmits from 570 mμ to red end
29	0	5.99	Transmits from 610 mμ to red end
34	0	4.70	Cuts out 470 mμ to 660 mμ
43	++	9.96	Cuts out 560 mμ to red end
47-A	0	2.94	Transmits from 400 mμ to 500 mμ
61	0	3.86	Transmits from 500 mμ to 570 mμ
63	0	1.42	Transmits from 490 mμ to 540 mμ
77-A	++	10.06	Transmits 540–560 mμ and 610 mμ on

A sensitized rabbit was also exposed to sunlight through filters 23-A and 29 alone. As in the case of irradiation by means of the apparatus in figure 1, filter 23-A allowed sunlight to evoke the symptoms of photosensitivity whereas filter 29 protected the animal. Since filter 29 allows almost all the infra-red to pass, this spectral region cannot be capable of causing hypericism.

Table 2 summarizes the results obtained from the experiments with carbon

are irradiation, and includes a short description of the characteristics of the filters used. Also given in the third column of table 2 are figures which express the predicted effectiveness of the light transmitted through each of the filters if hypericin were the photosensitizer. These values are only gross approximations. They were derived by obtaining for various wave lengths the product of the emission of the light source, the transmission of the given filter in the optical path, and the absorption coefficient of an aqueous hypericin solution buffered at pH 7.5. The area under the resultant curve was then measured. This calculation is described in detail by Blum (1). The energy distribution values for the light source were taken from the National Carbon Company Circular no. CP-1103, *Radiation characteristics of everyready sunshine and therapeutic carbons.* These can only be regarded as approximate values. The absorption spectrum of hypericin in the skin was assumed for the purpose of calculation to be that of hypericin in an aqueous buffered solution shown by curve B in figure 2. Although there are numerous inherent inaccuracies in this calculation, the predicted effectiveness of the various wave length regions agrees reasonably well with the observed effectiveness.

The three filters which pass light capable of producing a response, namely, 23-A, 43, and 77-A, have a predicted effectiveness of almost double the other filters used. These three filters transmit the region of the spectrum from 540 mμ to 610 mμ, hence the symptoms of experimental hypericism must be produced by visible light of this spectral region. The region of the spectrum from 400 mμ to 540 mμ exerts some effect on sensitized animals but not nearly to the same extent as the region 540 mμ to 610 mμ. The longer wave lengths beyond 610 mμ are apparently ineffectual in the production of symptoms of hypericism.

In all these experiments the ultra-violet was eliminated by filter 2-A since facilities were not available for a satisfactory study of this spectral region.

The wave length region 540 mμ to 610 mμ agrees fairly well with the region of maximum absorption of hypericin both in acetone and in aqueous solution buffered at pH 7.5 (fig. 2). Pace and Mackinney (3) have described the relatively wide variations in position and intensity of the absorption maxima of hypericin with changes of solvent. Hence, at present any more quantitative comparison of the action spectrum of hypericism with the absorption spectrum of hypericin in the skin awaits a better appraisal of the latter. From the data at hand it may be said that the action spectrum of experimental hypericism agrees approximately with the best available absorption spectrum of hypericin.

Oral feeding of hypericin. Pulverized hypericin was rubbed up with 0.5 per cent gum tragacanth solution in water, and the resulting suspension was diluted to contain 20 mgm. of hypericin per cc. The mixture was administered to two 180 grams white rats by stomach tube in divided doses over a period of six hours. One rat received 0.5 cc. every three hours, and the other received 1.0 cc. every three hours. Thus, the rats received a total of 30 mgm. and 60 mgm. respectively of purified hypericin. On the following day these two rats, together with two control animals, were exposed to sunlight through a window glass filter.

In five minutes a distinct erythema of the ears was noted in both of the

hypericin fed rats, and after ten minutes both animals were scratching vigorously and seeking shade. The control rats showed none of these typical symptoms of photosensitivity.

Thus, oral administration of hypericin can cause photosensitization in white rats with a dosage (not minimal) of 167 mgm. of hypericin per kilo of body weight.

DISCUSSION. There has been little doubt that hypericism was due to a combination of the ingestion of *Hypericum sp.* and subsequent exposure to light. It had also been shown that a pigment which can cause photosensitization when injected, i.e., hypericin, could be isolated from St. Johnswort. However, as has been repeatedly pointed out (1), (4), the mere isolation from a plant of a pigment which can cause photosensitization when injected is not conclusive evidence that the pigment is responsible for the natural occurrence of a photodynamic disease. Chlorophyll, for example, may be mentioned as a pigment which can cause photosensitization when injected but which has no effect when eaten. Furthermore, in the disease Geeldikkop certain plants cause photosensitization although they do not contain the responsible photodynamic pigment (1), (5). However, this type of photosensitivity is accompanied by extensive liver damage, and the livers of animals suffering from hypericism are normal.

Thus, before hypericin could be definitely established as the causal agent in hypericism it was necessary to demonstrate its presence in the skin of animals sensitized by feeding on the plant. It was necessary to show that oral administration of hypericin, when isolated from the plant, could produce photosensitization in order to demonstrate the effectiveness of the suspected pigment through the normal port of entry into the body. Finally, a determination of the action spectrum of the disease was undertaken in order to further define the photochemical receptor in the disease.

From the results of these experiments it may be stated that hypericism is caused directly by the ingestion of hypericin. The pigment is apparently absorbed unchanged and distributed throughout the body of the animal. The absorption of light by this pigment brings about destructive changes in skin irradiated with the wave lengths absorbed by the pigment. These changes produce the typical syndrome of the disease.

Blum and Pace (unpublished data) have shown that molecular oxygen is necessary for photodynamic hemolysis using hypericin as the sensitizer. All this evidence indicates that the sensitization to light in hypericism is photodynamic action, i.e., photo-oxidation of readily oxidizable substances in the skin (1).

SUMMARY

1. Hypericin, a red fluorescent pigment, has been identified spectroscopically in acetone extracts of various tissues from rabbits sensitized by feeding on *Hypericum perforatum*. Hypericin has also been demonstrated spectroscopically in the skin of a living rabbit sensitized in like fashion.

2. Administration of hypericin by mouth has been shown to cause typical photosensitization in white rats.

3. The region of the visible spectrum responsible for experimental hypericism has been found to correspond, within reasonable limits, to the principal absorption bands of the pigment hypericin in the visible region of the spectrum.

4. It is concluded that hypericin is the photosensitizer responsible for the production of the syndrome of hypericism in domestic animals.

Acknowledgments. The writer is indebted to Dr. G. Mackinney for his kindly criticisms and suggestions during the course of this work, and to Dr. H. F. Blum for introduction to the problem and in the preparation of this manuscript.

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WATER METABOLISM OF THE CHICKEN (GALLUS DOMESTICUS) WITH SPECIAL REFERENCE TO THE RÔLE OF THE CLOACA[1]

WILLIAM M. HART AND HIRAM E. ESSEX

From the Department of Physiology and the Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota

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The majority of workers have held that absorption of water from the cloaca is an essential part of avian economy[8, 12, 15], but the recent work of Hester, Mann and one of us (Essex) casts doubt on the validity of such a conclusion. A review of the literature makes it apparent that the conclusions of the earlier workers on the problem of absorption from the cloaca were drawn largely from qualitative and, for the most part, incidental observations.

The concept of absorption of water from the cloaca appears to have originated with Wiener and was amplified further by Milroy and later by Sharpe[12, 13]. The hypothesis was formulated from the observation that birds possessing an artificial anus drank more water than controls. Unfortunately, exact figures were not given by any of these workers, and later studies have shown that the daily volume of urine may be surprisingly small.

In the present paper we have recorded data on the total water balance of the chicken. Studies were made first on birds that had artificial anuses (procedure of Milroy) in order to repeat and extend the observations of previous workers. Following this type of surgical procedure the bird retains the possibility of some reabsorption, as the urine bathes the mucosa of the proctodeum before expulsion. Therefore, a technic was developed by which the ureters were brought directly to the outside. Following this type of surgical procedure, there is no mucosa left, other than that lining the ureters themselves, as possible surface for absorption.

Birds were brought into the laboratory and during control periods the daily consumption of food and water was determined. Subsequently, an artificial anus was created in some birds, while the ureters of others were exteriorized. After an adequate period of recovery, the consumption of food and water was studied again. The urine and feces were collected in separate receiving vessels and examined for amount and for content of water, uric acid and chloride.

METHODS. *Construction of the artificial anus.* Figure 1*a* shows the normal rectum and cloaca of the fowl. With the bird under pentobarbital sodium anesthesia it was secured on its back and a midline incision about 5 cm. long was made, starting about 16 mm. from the anal orifice. The rectum was severed at the desired point and the distal end closed at its junction with the cloaca.

[1] Abridgment of thesis submitted by Dr. W. M. Hart to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiology.

The rectum was brought through the body wall and sutured to the peritoneum with interrupted silk sutures. The skin was stitched to the peritoneum and drawn inward toward the new external opening of the rectum (fig. 1*b* and *c*).

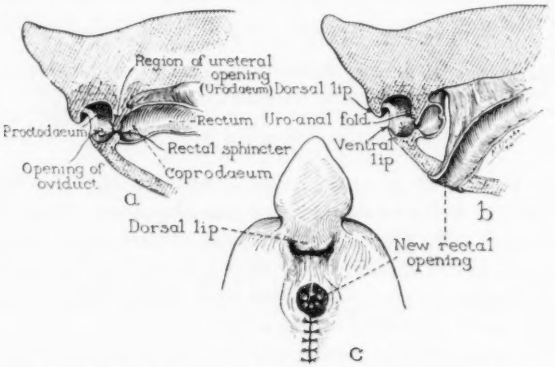

Fig. 1*a*. Sagittal section of the rectum and cloaca of the fowl; *b* and *c*, technic of construction of an artificial anus.

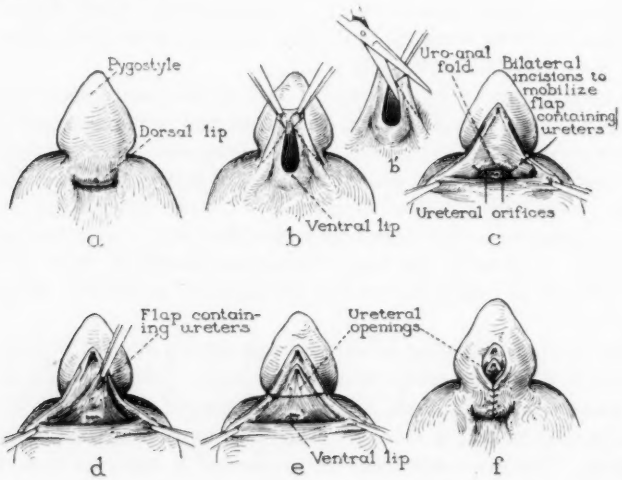

Fig. 2. The technic of exteriorization of the ureters of the fowl. See text for explanation of figure.

Procedure for exteriorization of the ureters. Figure 2*a* shows the region of the cloaca before operation. With the bird under pentobarbital sodium anesthesia the dorsal lip of the cloaca was pulled up by means of forceps as shown in figure 2*b*. Two cuts were made with the scissors on either side, directed inward and downward (fig. 2*b* and *b'*). The result of these two cuts was a triangular flap removed from the dorsal lip. This flap was brought high onto the pygostyle and

fixed to the skin by a silk suture (fig. 2*c*). The uro-anal fold was brought into view in this way. This fold (fig. 1) separates the urodeum from the proctodeum. The next step of the procedure, and perhaps the most difficult, was to free this fold with the ureters attached and bring it up onto the pygostyle. A cut was made on each side (fig. 2*c*) and the fold freed from the rectum below and the tissue above by blunt dissection. The flow of urine made it possible to locate the ureters and avoid cutting them. The freed fold was elevated (fig. 2*d*) and stitched to the triangular flap on the pygostyle (fig. 2*e*). The dorsal lip of the cloaca then was reconstituted by approximating the lateral portions remaining after removal of the triangular flap (fig. 2*f*). This closure was made with care, because some trouble was had with sloughing during the healing process.

A discussion of the anatomy of the cloaca which, in the interests of conservation of space we have omitted, may be found in the writings of Boyden (3, 4).

Procedure for collection of the urine. By means of a cannula of original design, urine was obtained from birds that had not undergone surgical procedures on the cloaca. This instrument consists of a 10 mm. glass tube about 9 cm. in length. Fused to one end of the tube is a glass ball about 23 mm. in diameter. A large hole is made in the glass tube very close to the ball; its edges are fire-polished and a small flange is turned outward. In the collection process, the ball is inserted into the cloaca and beyond the sphincter of the rectum. The hole in the tube then rests immediately beneath the urodeum. By clasping the ball the rectal sphincter holds the tube in place during the collection period.

Gauze-collodion cannulas were made for the collection of urine from the birds after operation. The cannulas were approximately 20 mm. in diameter and 30 mm. long and they were reinforced with a wire ring at the distal end. A toy balloon was slipped over this ring during the collection process. The proximal end of the cannula was sewed to the skin around the ureteral fistula by interrupted silk sutures. A continuous thread was used to draw the skin more closely about the cannula. In this manner a watertight connection was formed between the cannula and the skin. A harness of cloth was designed to hold the weight of the urine bag and to hold a square of oiled silk for the collection of feces. The birds were free to move about their separate cages in a normal way throughout the collection period. To accustom the birds to the use of the harness they were required to wear it for several days in advance of the collection period.

The estimation of uric acid was done by means of the colorimetric method of Benedict and Franke.

Determinations of the concentration of chloride in the plasma were done on 1 milliliter of plasma, using the modified Volhard-Harvey titration[11]; the same method was used for the urine. Determination of the pH of the urine was done with a quinhydrone electrode.

Physiologic Effects Produced by the Artificial Anus. None of the birds that had artificial anuses remained suitable for experimentation for more than about three weeks. After about three weeks the gut became atonic and even though the new anus was adequately patent, elimination was slow and obstruction in-

evitable. In spite of these difficulties, very satisfactory experimental birds were obtained and kept in good condition for two or three weeks. One hen (table 1) was kept on a continuous collection period of nine days. Its state of health was excellent during the progress of the experiment but a rectal obstruction developed about a week after this period ended.

Physiologic Effects of Exteriorization of the Ureters. Hester and associates reported a procedure for exteriorization of the ureters in which the dorsal portion of the anal ring was elevated onto the pygostyle. This type of operation has the disadvantage that the urine can run back into the coprodeum. In the method presented in this paper the urine is voided directly to the outside at all times without making contact with any appreciable mucosal surface.

TABLE 1

Water balance study on a hen (bird 1) that had an artificial anus; gut severed just proximal to proctodeum; 1 per cent salt in diet

DATE, 1940	BODY WEIGHT	FOOD INTAKE	WATER INTAKE	WET WEIGHT FECES	DRY WEIGHT FECES	WATER IN FECES	URINE VOLUME	pH OF URINE	URIC ACID IN URINE
	gm.	*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*cc.*		*gm.*
8–21	2,328	38	240	37	8	29	110	6.17	1.65
8–22	2,309	59	265	35	8	27	173	6.75	1.43
8–23	2,310	70	270	39	10	29	144	6.64	1.50
8–24	2,357	89	240	44	10	34	129	6.89	1.46
8–25	2,375	85	195	44	10	34	133	6.83	1.77
8–26	2,366	98	215	63	22	41	127	6.34	
8–27	2,343		250	57	16	41	103	6.34	
8–28	2,428	114	235	76	18	58	123		
8–29	2,404	143	270	74	18	56	115		
Averages.....	2,358	87	242	52	13	39	129		

After the ingestion of large amounts of water (200 cc.) the urine was observed to be watery with shreds of mucus and occasional blobs of uric acid. Birds ingesting smaller amounts of water excreted a less copious urine which often contained a high concentration of solid uric acid. In most birds the urine collected about the fistula as a gelatinous mass of uric acid. The feces became dry and assumed a form quite different from the usual.

If a bird was placed on the standard diet without added sodium chloride, a loss of body weight was observed in the first few days after the surgical procedure (fig. 3). After a variable length of time the hematocrit value was greater than normal and the concentration of chloride in the plasma was less than normal. If sodium chloride was not added to the diet, the bird continued to lose body weight until it died. Extreme loss of body water was apparent in the almost total loss of muscle volume, the deep sunken eyes and the black, shriveled comb. At this point concentrations of chloride were found as low as 328 mgm. per 100 cc. of plasma (normal 390 to 425 mgm. per 100 cc.) and hematocrit values as high as 60 per cent (normal 30 to 40 per cent).

If sodium chloride was added to the diet in any manner, the foregoing changes were prevented or corrected. One per cent salt in the food was sufficient to accomplish this result. In many cases drinking water containing 0.2 per cent sodium chloride was placed in the cage and the bird permitted to make its own choice between tap water and salt water. Intraperitoneal injections of Ringer's solution or physiologic saline solution also were used. When salt was given to the bird in any of these ways, a rapid and prompt response was obtained. The body weight increased rapidly; the hematocrit and plasma chloride values re-

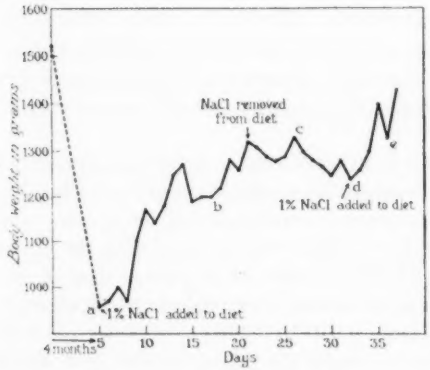

Fig. 3

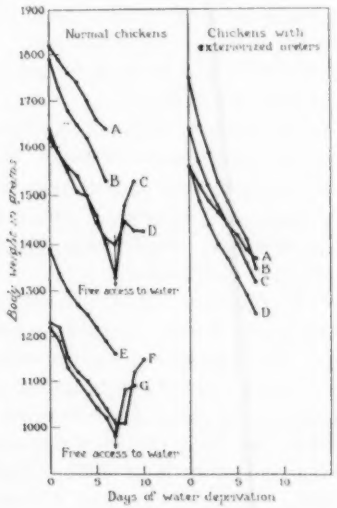

Fig. 4

Fig. 3. Restoration of normal body weight by the addition of 1 per cent salt to the food of a chicken (bird 9). *a*. Concentration of chloride 328 mgm. per 100 cc. of plasma, hematocrit value 54 per cent; *b*, concentration of chloride 416 mgm. per 100 cc. of plasma, hematocrit value 32 per cent; *c*, concentration of chloride 412 mgm. per 100 cc. of plasma, hematocrit value 32 per cent; *d*, concentration of chloride 410 mgm. per 100 cc., hematocrit value 43 per cent; *e*, hematocrit value 38 per cent.

Fig. 4. Loss of body weight when drinking water was withheld from control chickens and chickens having exteriorized ureters.

turned to normal almost immediately. If salt was withheld again, loss of weight occurred as before.

Bird 9 furnished the most complete data we obtained on these effects. After exteriorization of the ureters this hen was placed on a diet without added salt. The wound healed completely in five days but loss of weight was rapid and progressive. After six weeks the weight of this hen had decreased from the initial value of 1,300 grams to 800 grams. At this point its body water was extremely depleted. The concentration of chloride in the plasma was 332 mgm. per 100 cc. and the hematocrit value was 52 per cent. The concentration of

sodium in the plasma was 317 mgm. per 100 cc. (normal 372 mgm. per 100 cc.). Daily intraperitoneal injections of Ringer's solution were started at this point. Immediate clinical improvement was evident. The body weight increased steadily. One per cent salt was added to the diet the following month. After three months this bird exceeded its original body weight by 30 grams. It was kept in good physical condition, as evidenced by the fact that it started a period of egg laying about five months after the operation. At the beginning of the ninth month of observation it was placed on the basal diet without added salt for four months (fig. 3). At the end of this time its body weight was 960 grams. The concentration of chloride in the plasma was 328 mgm. per 100 cc. and the hematocrit value was 54 per cent. One per cent salt then was added to the diet. The response was immediate and rapid. After six weeks the bird had regained its original body weight.

We performed numerous similar experiments which demonstrated that the responses just described were characteristic. Birds that have exteriorized ureters may be kept indefinitely and in excellent health if due attention is paid to chloride requirements. Many of our birds entered on prolonged periods of egg production. Bird 9 was still living and in excellent health nearly eighteen months after operation.

Water Balance. Many of the early workers noted tremendous increases of consumption of water after the construction of an artificial anus or after hepatectomy. If absorption from the cloaca occurs in significant amounts, one would expect an increase of consumption of water to follow interference with the normal anatomic relations. Therefore, we studied this point with a great deal of care.

Birds that have not been operated on consume a variable amount of water. Their twenty-four hour intake ranges from 0.070 to 0.175 cc. of water per gram of bird. Therefore, it was necessary to study the water intake and weight fluctuations of the same birds before and after surgical procedures.

There was a temporary increase of the water intake of some but not of all birds that had artificial anuses (tables 2 and 3) immediately after the operation but the intake when increased returned to the control quantity a short time later.

After exteriorization of the ureters, five birds of the 150 in this group increased their intake of water four or five times the basal value. As a rule, however, the increases were not more than a few cubic centimeters (tables 4 and 5). Some birds increased their intake of water intermittently. When permitted to drink salt water, the birds nearly always but not invariably increased their total intake of fluid. When birds that had exteriorized ureters were fed a diet without added salt, they always increased their consumption of water (table 6). The water intake was often double that of the period when salt was added to the diet.

For purposes of studying the normal volume of urine of birds that had exteriorized ureters we necessarily used birds receiving adequate salt intake (1 per cent sodium chloride in the food). Under these conditions birds that had exteriorized ureters and birds that had artificial anuses behaved exactly alike. The volume of urine varied from 50 to 180 cc. or 28 to 70 per cent of the water intake for the day (tables 1 and 7). This percentage of the intake was fairly constant for any given bird.

TABLE 2

Daily consumption of food and water and fluctuation of weight in chicken 2 before and after creation of an artificial anus; 1 per cent salt in diet

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE DAILY WATER INTAKE	AVERAGE DAILY FOOD INTAKE	GM. FOOD / GM. BIRD	CC. WATER / GM. BIRD	CC. WATER / GM. FOOD	COMMENT
	gm.	*cc.*	*gm.*				
8-10 to 8-14	2,578	270	143	0.055	0.105	1.89	
8-16							Artificial anus created. Blood Cl: 394 mgm. per 100 cc. Hematocrit reading: 27 per cent
8-17 to 8-23	2,338	250	55	0.024	0.107	4.55	
8-24 to 8-30	2,388	236	113	0.047	0.099	2.09	Hematocrit reading: 32 per cent

TABLE 3

Daily consumption of food and water and fluctuation of weight in chicken 3 before and after creation of an artificial anus; 1 per cent salt in diet

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE DAILY WATER INTAKE	AVERAGE DAILY FOOD INTAKE	GM. FOOD / GM. BIRD	CC. WATER / GM. BIRD	CC. WATER / GM. FOOD	COMMENT
	gm.	*cc.*	*gm.*				
6-17 to 6-23	1,613	213	93	0.058	0.132	2.29	
6-25							Artificial anus created
6-26 to 7-2	1,596	238	82	0.051	0.149	2.90	
7-3 to 7-9	1,633	300	58	0.036	0.184	5.17	
7-10 to 7-15	1,542	115	40	0.026	0.075	2.88	Blood Cl: 406 mgm. per 100 cc. Hematocrit reading: 35 per cent

TABLE 4

The daily consumption of food and water and fluctuation of weight in chicken 4 before and after exteriorization of the ureters; 1 per cent sodium chloride in diet

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVERAGE FOOD INTAKE	GM. FOOD / GM. BIRD	CC. WATER / GM. BIRD	CC. WATER / GM. FOOD	COMMENT
	gm.	*cc.*	*gm.*				
5-6 to 5-10	1,317	231	106	0.080	0.175	2.18	
5-16							Ureters exteriorized
5-17 to 5-23	1,180	210	70	0.059	0.178	3.00	
5-25 to 5-31	1,255	235	89	0.071	0.187	2.64	
6-18 to 6-23	1,207	122	44	0.036	0.101	2.77	

The amount of water in the feces varied from 5 to 25 per cent of the water intake. This figure, too, was fairly constant for any given bird.

Bird 1 (table 1) may be used to illustrate the calculation of the water balance.

From August 21 to August 26 inclusive, this bird ingested 1,864 grams of food and water. It eliminated 1,078 grams of feces and urine in this period. The difference of these two figures is 786 grams. However, 38 grams of body weight was gained by the bird during the six days. Therefore, 748/6 gives 124.7 grams per day lost by way of the respiration (that lost through the skin is doubtless negligible). Values ranging from 110 to 144 grams may be found by similar calculations from the data given for other birds.

TABLE 5

The daily consumption of food and water and fluctuation of weight in chicken 5 before and after exteriorization of the ureters

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVERAGE FOOD INTAKE	AVERAGE NaCl INTAKE, 0.2%	GM. FOOD / GM. BIRD	CC. WATER / GM. BIRD	CC. WATER / GM. FOOD	COMMENT
	gm.	*cc.*	*gm.*	*cc.*				
4-1 to 4-9	1,568	155	69		0.044	0.099	2.25	
4-16								Ureters exteriorized
4-20 to 4-26	1,507	106	78	129	0.052			
4-27 to 5-4	1,504	178	69		0.046	0.118	2.58	
5-6 to 5-13	1,523	199	74		0.049	0.131	2.69	Blood Cl: 400 mgm. per 100 cc. Hematocrit value: 38 per cent

TABLE 6

The daily consumption of food and water and fluctuation of weight in chicken 6 before and after exteriorization of the ureters; no salt added to the diet

DATE, 1941	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVERAGE FOOD INTAKE	GM. FOOD / GM. BIRD	CC. WATER / GM. BIRD	CC. WATER / GM. FOOD	COMMENT
	gm.	*cc.*	*gm.*				
1-27 to 2-3	2,209	100	78	0.035	0.045	1.28	
2-4							Ureters exteriorized. Blood Cl: 400 mgm. per 100 cc. Hematocrit value: 40 per cent
2-14 to 2-20	2,080	213	48	0.023	0.102	4.44	
2-21 to 2-26	2,042	250	70	0.034	0.122	3.57	

In calorimetric studies on hens, Barott and associates found the water eliminated by way of the respiration to be 2.8 ± 0.1 mgm. per hour per gram at the basal level and 3.1 ± 0.1 mgm. per hour per gram during eight hour periods between 2 p.m. and 10 p.m. These figures give 154 cc. at the basal level and 171.1 cc. for the eight hour period as twenty-four hour values for a bird of 2,300 grams.

Withdrawal of Drinking Water from Birds That Had Exteriorized Ureters. The

TABLE 7

Water balance study on chicken 7 which had exteriorized ureters; 1 per cent salt added to the diet

DATE, 1940	BODY WEIGHT	FOOD INTAKE	WATER INTAKE	WET WEIGHT FECES	DRY WEIGHT FECES	WATER FECES	URINE VOLUME	pH OF URINE	URIC ACID	PLASMA Cl.	HEMATOCRIT VALUE
	gm.	*gm.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*		*gm./100 cc.*	*mgm./100 cc.*	
8–21	1,418	61	220	35	5	30	100	6.71	1.56	412	40
8–22	1,419	85	255	47	12	35	112	7.29	1.09		
8–23	1,484	90	245	74	13	61	107	6.64			
8–24	1,480	96	250	63	4	59	144	6.95	1.36		36
Averages....	1,450	83	242	54.7	8.5	46.2	115.7				

TABLE 8

Collection, by use of the glass cannula, of urine from normal bird (no. 8) prior to operation; 1 per cent salt in diet

TIME	URINE VOLUME	COMMENT
	cc.	
11:14		Clear, watery urine
11:28	7.5	Milky urine
11:30		White shreds appear
11:40		Bird moderately disturbed
11:43		Urine less cloudy
11:53	3.0	Urine clear as water
12:00		Urine slightly cloudy
12:10		Urine less turbid; bird moderately disturbed
12:30		Urine clear as water
12:32		Urine cloudy again, with chunky material
1:00		Anuric for past 28 minutes; asleep
1:10		Flow increased to 2 drops per minute
1:20	2.5	Collection ended
	13.0	Total volume of urine collected in period
		Two days after foregoing collection
1:15		Urine slightly turbid; 3 drops per minute
1:35	3.0	Urine very milky
1:41		Urine clear as water; bird struggles
1:44		Urine cloudy
1:47		Urine clear as water; bird disturbed
1:53		Urine clear as water; 4 drops per minute
1:58		Urine slightly turbid; 1 drop per minute
2:00		Urine thick white; 1 drop per minute
2:19		Anuric for last 14 minutes
3:10	6.5	Collection ended
	9.5	Total volume of urine collected in period

importance of absorption of water from the cloaca or rectum should be demonstrated by the ability of the fistulous bird to withstand a prolonged lack of water. Accordingly, birds that had exteriorized ureters were refused water and their loss of weight was compared with that of control birds under the same situation (fig. 4). Losses of weight should be compared during the first few days of observation while the birds are normally hydrated. Total losses of weight during the first three days of observation for the controls were 74, 78, 99, 102, 113, 120 and 121 grams. Simultaneous losses for the fistulous birds were 122, 132, 137 and 189 grams.

Urine Collections From Hens Before Surgical Procedure. Hester and associates, using the funnel technic of collection of urine (5), observed a primary phase of copious flow of urine followed by a phase of scant flow of urine. They showed that nervous influences are among the factors which determine the quantity of flow of urine. We have extended these observations and confirmed the conclusions of the previous work. The cannula described in this paper for collecting urine permitted a longer period of observation than did the funnel technic. A typical experiment will now be presented briefly.

One of the birds secreted 7.5 cc. of urine in the first fourteen minutes of collection. In the next twenty-five minutes it secreted 3 cc. of urine, and in the following eighty-seven minutes it secreted 2.5 cc. of urine. In the primary phase the urine secreted was clear and watery while the urine collected during the secondary phase was reduced in volume and contained solids in varying amounts. On a different day the same bird secreted 3 cc. of urine in the primary phase of twenty minutes. In the following period of ninety-five minutes it secreted 6.5 cc. of urine (table 8).

COMMENT. It is clear that studies on water consumption are not adequate criteria for establishing the concept of absorption from the cloaca.

The excessively large estimates placed on the urine volume (600 to 1,500 cc. per 24 hrs.) by some previous workers may be accounted for in the following ways. Wiener and Milroy were studying birds after removal of the liver. Minkowski gave a satisfactory explanation for the increased water intake after this operation by pointing out that more blood was required to go to the kidney, which should result in increased excretion of urine.

Davis found that anesthesia caused a greatly increased flow of urine and he pointed out that calculations of the daily volume of urine under such conditions were erroneous. The observations of Hester and associates of a diuretic phase of secretion of urine indicate an additional reason why a false impression of the daily volume of urine would follow if calculations were based on the data obtained from short collection periods.

Davis suggested that the bird depends on absorption from the cloaca for the conservation of various urinary constituents, including creatine, urea and chloride.

In our studies of hens that had exteriorized ureters, we found that the addition of sodium chloride to the dietary would maintain them in excellent health for indefinite periods. Because of the latter fact and because severely deficient

birds could be restored to normal by giving salt alone, we conclude that reabsorption of electrolytes and water from the rectum is the only mechanism of importance in the normal economy of the chicken.

When drinking water is withheld, fistulous birds lose weight more rapidly than controls. This observation constitutes suggestive evidence for the importance of the rectum in water conservation. Although we have these facts at hand, and although we have demonstrated the importance of absorption of chloride, our data do not permit us to make a final statement concerning the magnitude of these absorptions. Further investigation will be necessary to determine the type of work involved in the process of absorption.

Birds that have exteriorized ureters lose weight rapidly immediately after the operation. But fistulous birds that have been maintained on salt for long periods lose weight more slowly when placed on a diet without added salt. One bird lost nearly half its body weight in six weeks immediately after exteriorization of its ureters, but four months were required (fig. 3) to produce the same loss of weight after it had been carried on adequate salt intake for six months. Thus it would appear that there is some adaptation to the new condition.

Korr[7, 8] presented good evidence to show that absorption does not occur from the cloaca. We do not have data on this point, since our birds that had artificial anuses were given 1 per cent salt in the diet. This amount of salt is sufficient to maintain the fistulous bird and therefore would be ample for birds that had artificial anuses.

Korr found that 60 to 100 cc. of water, as a maximal amount, was excreted by the kidney for every gram of uric acid. These figures were based on determinations of total nitrogen of relatively dilute urines. From the data of Szalágyi and Kriwuscha we calculate 100 to 200 cc. of urine water for each gram of total nitrogen (chickens). We found 30 to 165 cc. of urine water to be excreted with each gram of uric acid. Since all our birds were ingesting normal amounts of water, the volumes of urine were doubtless normal.

SUMMARY

The daily consumption of food and water and weight fluctuations of normal birds were studied. Following this control period, artificial anuses were created in some of the birds while the ureters of others were exteriorized.

There was an increase of the water intake of some birds that had artificial anuses immediately after the operation but the intake returned to the control quantity a short time later.

When the fistulous birds were given 1 per cent salt in the diet, occasional increases of water intake were noted. If salt was not added to the diet, an increase of water intake always occurred. We do not have any satisfactory explanation for this paradox.

When salt was omitted from the diet of fistulous birds, weight losses were pronounced and eventually death occurred, with evidences of extreme depletion of body water. However, these effects were prevented or ameliorated by the

addition of 1 per cent salt to the diet and this amount of salt was sufficient to maintain the birds indefinitely.

Fistulous birds lose weight more rapidly than controls when drinking water is withheld, indicating that normally some water is reabsorbed from the rectum.

When adequate salt intake was assured, birds that had exteriorized ureters behaved exactly like those that had artificial anuses. The normal volume of urine varied from 50 to 180 cc. per day. The amount of water lost in the feces was usually about 10 per cent of the water ingested for the day. Vaporization from the lungs accounted for 110 to 144 cc. of water per day.

Each gram of uric acid eliminated was accompanied by 30 to 160 cc. of water.

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THE VASOMOTOR COMPONENTS IN THE VASCULAR REACTIONS IN THE FINGER TO COLD[1]

ALRICK B. HERTZMAN AND LAURENCE W. ROTH

From the Department of Physiology, St. Louis University School of Medicine, St. Louis, Missouri

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The vascular responses in the finger to local chilling have become well-known since their description by Lewis (1) and Grant (2). They usually occur in the following sequence: initial constriction occurs on application of the cold; some 5 to 16 minutes later dilatation occurs while the cold is continued; this reaction has a variable duration being followed by constriction and again by a second reactive dilatation, the cycle being repeated if the cold is continuous; on ending the cold, the vessels dilate rapidly so that the temperature of the previously chilled skin may considerably exceed that of a control finger.

These reactions were elicited also in the toes, ear, tip of nose and chin. Since these skin areas as well as the finger contain a large number of arterio-venous anastomoses, it was suggested that these vessels are the ones responsible for the reactive dilatation to cold.

The dilator reactions during cold persisted after sympathectomy and after degeneration of the sympathetic fibers. They disappeared after degeneration of the sensory fibers but not after section of the latter. This similarity to the histamine flare suggested that the reactive dilatation is elicited by the liberation of H-substance which in turn excites the axone reflex.

This description is based on observational methods which do not clearly indicate the time relations of vasomotor activity to the reactions to cold. It appeared probable that suitable plethysmography would examine these reactions in greater detail and would permit a better correlation between the reactions and the vasomotor reflexes elicited by the cold. It also seemed possible that through plethysmographic analysis one would obtain additional information on the relative participation of arterio-venous anastomoses, small arteries and arterioles, and larger artery trunks (e.g., the digital arteries), in the constrictions and reactive dilatations to cold in the human finger.

METHODS. The vascular reactions in the finger to local cold were followed by means of the photoelectric plethysmograph (3). The use of this instrument simplified the mechanical problem of applying cold and of recording simultaneously the changes in volume or in volume pulse. Variations in the latter were considered as indicative of changes in arterial tone.

Cold was applied to the finger in three ways: 1, cold water was circulated about

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the finger; 2, the finger was placed in a bath of chopped ice; 3, cold water was circulated through a copper tube soldered to a thin copper sheath in contact with nearly the entire surface of the finger. In each case the photoelectric plethysmograph was applied to the pad of the finger being chilled.

When the finger was immersed in water, the plethysmograph was protected by cementing a sheet of glass over the openings of the plethysmograph tubes which were passed through a rubber stopper in the wall of the bath. The finger dipped into the bath from above. It was kept in place over the plethysmograph by a piece of sponge rubber cut to fit between the finger and the wall of the bath without exerting significant pressure on the finger. Since the top of the bath was open, there was no mechanical interference with the circulation in the finger.

The copper sheath used to chill the finger was soldered directly to the plethysmograph. This was the most convenient means of chilling the finger. It was effective and provided the same results as the immersion method.

A finger on the same hand and often also one on the other hand served as controls. Temperatures of the pads of the control and experimental fingers were continuously recorded by thermocouples.

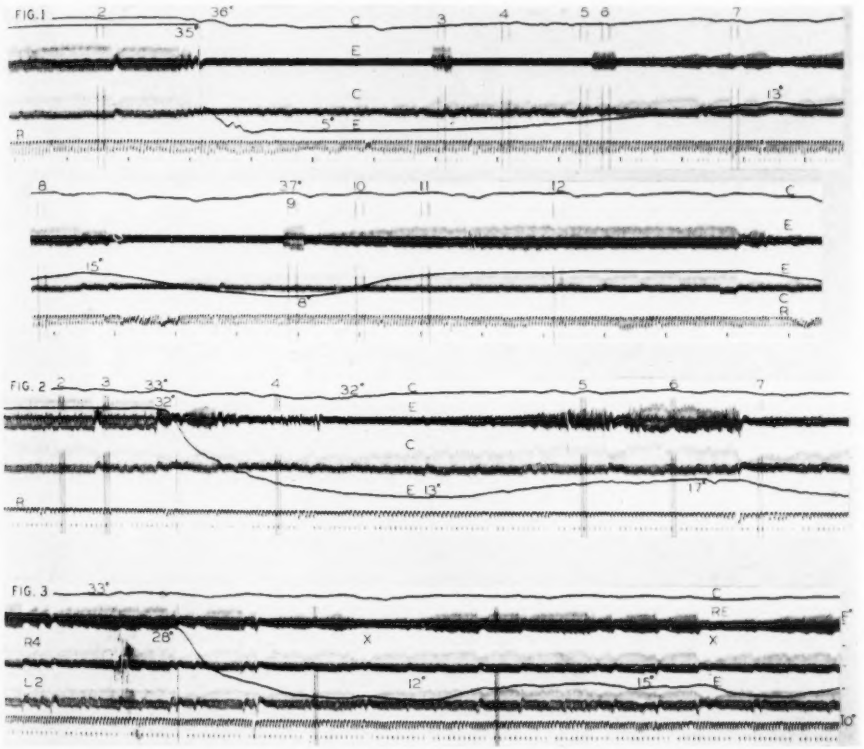
Every effort was made to secure the comfort of the subject who rested easily on a cot in a semi-reclining position. However, in prolonged experiments of this type, the continuation of the same position of the arm often results in numbness in fingers, hand and forearm. As far as we could detect, this did not directly affect the finger blood flow and the vascular reactions. We saw no relation between the onset and the degree of numbness and the finger blood flow.

The subjects were medical students in good health and with normal circulatory systems, unless otherwise noted. Fitting the apparatus to the subject and similar preparations usually required thirty to forty-five minutes during which time the subject was resting on a cot. Hence, a control record of fifteen to twenty minutes came after a considerable rest period so that the subject was probably in good equilibrium with the room climate (25°–27°C.) before experimenting began. Attainment of equilibrium was favored by the subject having been in the building for several hours before coming to the laboratory.

A serious difficulty with prolonged experiments of the type reported here is that immobilization of the subject results in a slow progressive decrease in the blood flow in the finger. This may not show well in the finger temperatures when the room temperature is in the range of 25° to 27°C. The cooling effect of this air temperature on the finger is slight, hence it is possible for a considerable reduction in blood flow to occur without much effect on the finger temperatures. This is more apparent when the flows are high than when they are low. The effects on finger temperature are more obvious at lower air temperatures. This progressive fall in finger flow was noted by Lewis (1) and allowed for in comparing the reactions of the chilled finger with the control after the application of cold had ended. His experiments were carried out at lower room temperatures.

RESULTS. *The initial constriction.* On application of cold to the finger (fig. 1–5), immediate and profound constriction occurs not only in the finger being

chilled but also in the control fingers of the same and opposite hands. The constriction in the chilled finger may be immediately complete (figs. 1 and 4) or it may become progressively more intense within a brief period of ten to



Figs. 1–3. Effects of local cold in finger circulation. Records of volume pulses and temperatures of experimental or chilled finger, *E*, and of control finger, *C*. Wave form records taken at triple vertical signals (see following paper). Time in minutes and 5 seconds. Respiration by pneumograph.

Fig. 1. Chopped ice applied at break in temperature record. Cold continued for remainder of record. Amplification temporarily increased at *3*, *6* and *9* for wave form records. R.T. 26°C. Male. Age 24. B.P. 112/74.

Fig. 2. Ice water at break in temperature record. Cold continued. R.T. 25.5°C. Male. Age 23. B.P. 120/80.

Fig. 3. Ice water at break in temperature record. Cold continued. R.E.—right index finger (experimental or chilled); R4—right fourth finger; L2—left index finger. R.T. 26°C Male. Age 24. B.P. 120/74.

seventy seconds (fig. 2). The initial constriction in the chilled finger may be brief (60 sec.) or it may last longer, (4 to 15 or more min.). The initial constrictions in the control fingers are usually less intense and of shorter duration. They are due to vasoconstrictor reflexes elicited by the cold since

they appear in all control fingers (of the same and opposite hands) at the time they occur in the chilled finger.

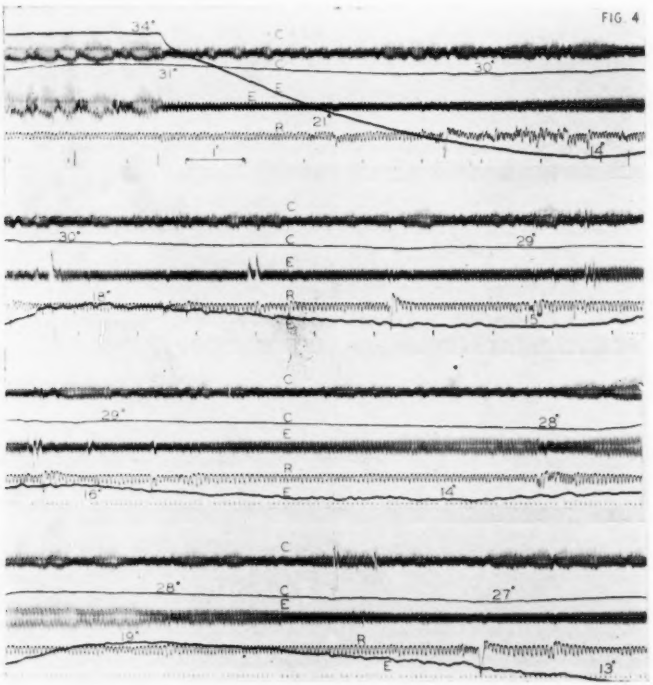


Fig. 4. Experiment illustrating paralysis of vasomotor fibers in the chilled finger during the reaction of dilatation. Cold applied by cold water (4°C.) flowing through a copper applicator. Volume pulses and temperatures of experimental, *E*, and control, *C*, fingers. Cold applied at break in temperature record. Time in minutes and 5 seconds. Respiration by pneumograph. R.T. 25°C. Male Hawaiian. Age 23. B.P. 98/58. Subject dislikes cold very much but does not show cold allergy.

Fig. 5. Comparison of the effects of the cold pressor test (at horizontal bar) and of local cold (ice at break in temperature) on the finger circulations. Volume pulse and temperature records from experimental, *E*, and control, *C*, fingers of same hand. R.T. 26°C. Male. Age 24. B.P. 116/78. Normo-reactor.

The differences in the intensity and duration of the initial constrictions in the chilled and control fingers may be due to a direct local constrictor action of cold on the arteries superimposed on vasoconstrictor reflexes elicited by the cold or to these reflexes acting exceptionally strongly on the chilled finger.

The changes in finger temperature are pertinent to a decision. The initial constriction in the chilled finger may proceed to obliteration of the pad pulse before there has been any large fall in the recorded temperature of the pad of the chilled finger (fig. 4). In some cases, the chilled finger's pad temperatures were as high as 30°C. at the time of complete constriction. It may be argued that the *surface* temperature of the finger tip does not represent the temperatures of the pad arteries whose reactions are being recorded and is therefore not significant as a basis for correlating the temperature drop with the intensity of the constriction. There seems to be no way of eliminating this error since the argument would apply equally well to measurement of the temperature of any part of the finger's surface. The comparison of the finger and forehead reactions to local cold (described in a following paper) suggests that the cooling of the finger arteries proceeds more rapidly than one would infer from the finger tip temperatures. The initial constriction due to a vasomotor reflex would favor swift cooling. Absence of this reflex in the forehead skin would prevent a rapid fall in temperature there. Arterial constriction would then proceed much more gradually.

The fall in temperature may not induce a sustained constriction in the chilled finger (fig. 3). The record of its volume pulses may parallel quite closely the records of the control fingers. Each vasomotor discharge to the latter may be faithfully reflected in the record from the chilled finger. Yet, the constrictions in the latter tend to be more prolonged (fig. 3 at *X*). It is difficult to believe that as a result of chilling, the vasomotor discharges would be selectively prolonged with respect to the cold finger. Two alternatives remain: either the vasoconstrictor effect is prolonged by the direct action of cold on the arteries or the lower temperature prolongs the life of the chemical mediator. Observations recorded below on the nature of the reactive dilatation bear on this question.

That the onset of the initial constriction is exclusively due to a vasomotor reflex is indicated by several observations: 1. The constrictor response of the sympathectomized toe (in a hypertensive patient) to cold resembled that of the forehead skin. Constriction developed gradually as the toe temperature fell. 2. Variability in the participation of vasoconstrictor reflexes in the constriction to cold was interestingly illustrated in a young man in whom a peripheral vascular disorder was suspected (skin from mid-calf to toes became fiery red then cyanotic on standing). The big toe showed, on first observation, the absence of vasoconstrictor reflexes to such procedures as psychic stimuli and deep breaths which were very effective in inducing constriction in the finger. Application of cold to the toe failed to elicit the initial constriction there. The cold stimulus was effective in eliciting vasoconstrictor reflexes since the finger constricted markedly when the cold was applied to the toe. As the temperature of the latter fell, the toe vessels constricted gradually but not to obliteration of the volume pulse. A reactive dilatation followed this constriction. Three days later during which time there was no therapy, the experiment was repeated. The toe now showed very effective vasoconstrictor reflexes and responded to a similar cold application with a typical immediate profound constriction which also occurred in the toes of the other foot. The toes were warm (about 31°C.)

and showed large volume pulses on each day so that the differences in behaviour to the cold and other stimuli were not due to differences in the blood supply. We have not had the opportunity to study this patient further. 3. The possible hypersensitivity of the finger arteries to cold in Raynaud's disease offered opportunity to compare the constrictions elicited by local cold with and without participation of vasoconstrictor reflexes (as indicated by the reaction of the control finger). Two experiments were done on a woman (age 40) in the earlier stages of the disease. In the first experiment, water at 15°C. was passed through the cold applicator attached to the right index finger. Constriction began very slowly and gradually about a minute later in this finger. This constriction became progressively more intense, reaching its maximum about fourteen minutes after the onset of cold. It was maintained for the duration of the cold (25 min.). There was no accompanying constriction at any time in the control finger. Both fingers were free of "spontaneous" waves during the entire observation. This subject gave an intense response in the fingers to the "cold pressor test". The absence of any indication of vasomotor participation in this response to local cold points to a direct action of cold on the finger arteries. This response was analogous to that occurring in the forehead. Repetition of the experiment at a later date with colder water (8°C.) showed the usual vasoconstrictor reflexes on the application of cold. The following reactive dilatation was remarkable for its size and duration but did not result in a subsequent swelling of the finger. 4. Similar experiments on another case of Raynaud's disease on which a unilateral cervical sympathectomy had been done, illustrated most convincingly that the absence of the vasoconstrictor reflexes in the chilled finger delays the onset of the constriction to cold and results in a more gradual development of the constrictor response.

It is interesting to note that these initial constrictions in the control fingers may be as intense as those elicited by plunging the opposite hand into ice-water (fig. 5). The constrictions may be better maintained in the latter case. Not all subjects give maximal constrictions in the finger on applying cold to it or to its neighbor, nor do they do so when the "cold pressor test" is done. It would be of interest to attempt a comparison of the finger constrictions in the normo- and hyper-reactor groups of subjects on the local application of cold and on making the "cold pressor test."

As the application of cold continues, the vessels in the control fingers usually relax partially while those in the chilled finger usually constrict still more as its temperature falls. That vasomotor activity in the control fingers continues at a higher level is indicated by the reduction in the average amplitude of the volume pulses, by greater frequency of the "spontaneous waves" as compared with the control period, and by the tendency of the control finger temperatures to fall.

It is difficult to account for the increased vasomotor activity on a thermosensory basis, for it continues after the chilled finger has become numb. Certainly the extremely small return of chilled blood from a single cold finger can have no direct central effect on the temperature regulating mechanism. The

vasoconstrictor effects of cold may be elicited without sensation. Thus, the blood pressure raising reflex from the cold pressor test was obtained in a patient showing hysterical anesthesia to cold, heat and pain in the cutaneous areas supplied by spinal segments (4). The patient was unaware of the application of ice to the forearm and hand; yet a significant rise in blood pressure occurred. Similarly, reflex vasodilatation may be obtained by warming an occluded limb in which thermal sensation is absent (5). The thermal sensations are therefore unnecessary to the appearance of vasoconstrictor reflexes in response to chilling or warming the skin. Neither is pain essential to their continuance.

The "reactive dilatation". Sooner or later (3 to 8 min. or more), after the beginning of the cold application and while the cold is continued, the constricted chilled finger vessels slowly and progressively dilate as indicated by the rise in the finger's temperature and by the increase in its volume pulse. The increase in the latter is usually obvious for several minutes while the temperature is still falling (fig. 4—upper record). This progressive dilatation is often limited to the chilled finger although it is also frequently preceded by dilatation in the control finger. This suggests that a decrease in vasomotor activity may be a factor in the dilatation.

The relation of vasoconstrictor reflexes to the reactive dilatation. Our experiments have permitted an examination of this relation. In some cases, vasoconstrictor reflexes were ineffective in the chilled finger during the "reaction," while in other experiments the opposite was true. In still other instances, reflex vasoconstrictor effects in the chilled finger developed slowly and tended to be prolonged. There were no systematic differences in the magnitude of the reactive dilatation in different subjects in relation to the absence of or presence and degree of vasoconstrictor reflexes in the chilled finger.

During the reactive dilatation in the cold finger, heightened vasomotor tone and spontaneous constrictions (vasomotor in origin) in the control fingers may be without any measurable effects on the chilled finger (fig. 4) even when its volume pulse is well above the control level. The "reaction" may develop into a large dilatation while high vasomotor tone continues in the control finger. Deliberately eliciting vasoconstrictor reflexes during the "reaction" as by plunging the opposite hand into ice-water, or by effective psychic and auditory stimuli, or by a deep breath, were also ineffective in the chilled finger in these instances, although strong constrictions were obtained in the control fingers. We interpret such results as indicating a temporary paralysis of the vasomotor fibers in the chilled fingers.

In searching for reasons for this "paralysis," we have examined its correlation with the temperature of the finger pad without success. "Reactions" occurred at finger pad temperatures between 5° and 23°C. "Paralysis" was seen with the finger pad temperatures as high as 29°C. as the result of a large "reaction" and was absent at 5°C.

The "paralysis" tended to disappear as the "reaction" developed (fig. 4—lowest record), but it might still be present when the volume pulse was again as large as or larger than during the control period. The "paralysis" might

continue throughout a "reaction" and the latter would then subside gradually into another prolonged constriction without evidence of vasoconstrictor activity (fig. 4—first and second "reactions"). The presence of "paralysis" bore no systematic relation to the size of the "reaction". But high vasomotor activity in the control fingers did limit the "reactions" in the cold finger if the vasoconstrictors were still active there (figs. 1 and 2), the reactive dilatation usually being ended by a vasoconstrictor discharge to all fingers. This point probably would be missed in temperature records since a fleeting constriction in the control finger too brief to influence noticeably the finger's temperature may be the signal for the onset of another prolonged constriction in the experimental finger (figs. 1 and 2). Continuous records of the volume pulse or of finger volume are required to illustrate the point.

The first constriction which occurs during the reactive dilatation may not pass into another prolonged constriction with a marked fall in finger temperatures. Several constrictions may occur before this happens (figs. 1 and 2). But it is usually true that the first considerable constriction in the control finger during the "reaction" ends the latter. In this sense, the duration of the "reaction" is limited by vasomotor activity.

The events elicited by the initial application of cold are now repeated. The constriction in the cold finger lasts for a variable period (4 to 8 min.). It is followed by another reactive dilatation which also is ended by a vasoconstrictor discharge (fig. 1). Several such cycles may occur providing the patience of the subject permits the prolonged sitting which is required.

The vasomotor "paralysis" is not always complete and it appears to pass through transitional stages which show in a constrictor response which develops gradually rather than in the abrupt manner usually true for the control fingers (last record of fig. 5); the response may also be much less intense and may last longer than in the control fingers. As the reactive dilatation proceeds, the constrictor response in the experimental finger becomes more and more like those in the control fingers: abrupt, intense, brief or long as may chance to be the case in the latter. One is reminded of the possibility of a chemical mediator being liberated at a slower rate, acting more slowly, and being more slowly eliminated due to the lower temperatures. Or, these effects may be due to the direct effects of the lower temperatures on the smooth muscle of the arteries.

Not all experiments progress in the manner described above. Some normal subjects fail to give prolonged constrictions to chilling the finger (fig. 3). Such subjects' fingers appear to have excellent blood supplies (judging by the amplitude of their finger volume pulses) which apparently prevent most effectively the fall in finger temperature required to bring on a prolonged constriction. After an initial constriction of brief duration in the experimental and control fingers, circulatory conditions, except for the lower temperature of the chilled finger, are restored to control levels and remain so. Inspection of the records in these cases often permits no distinction between the control and the experimental periods. These subjects may experience pain from the chilling. However, their discomfort is short-lived. In this respect they do not differ from the

subjects who present the cycle of prolonged constriction followed by the reactive dilatation.

Recovery. The period of recovery from the exposure to cold proceeds as described by Lewis (1). A reactive dilatation occurs in the chilled finger if there has been constriction from the cold. This "reaction" may not increase the flow above the control level but the fact that it is occurring is indicated by comparison of the pulses in the experimental and control fingers. The pulses in the former are larger.

There are several points which bear on the reasons for this dilatation during recovery in the experimental finger. First, it occurs even when there is a high vasomotor tone in the control fingers as indicated by the reduction in their volume pulse. Second, at this time the vasoconstrictor reflexes are as effective in the experimental finger as in the control fingers. The constrictions are as abrupt, and as intense in the experimental finger as in the control. Still the vessels in the former remain widely dilated while those in the control fingers are partly constricted. The differences in the circulatory situations may be quite impressive at times.

COMMENT. Interpretation of the experiments described above is concerned with two familiar phenomena: 1, the constrictor effect of local cold; 2, the reactive dilatation which follows while the cold application is continued and which is commonly recognized in the sensations of throbbing and of warmth in the exposed fingers.

1. The initial constriction in the skin of the finger due to local application of cold results from vasomotor reflexes elicited by the cold. This is indicated by the simultaneous constrictions occurring in the control fingers. Although differences in the intensity of the initial constriction in the experimental and control fingers are no more striking than those seen in responses to other stimuli, they are regularly exhibited. We take this to mean that an additional direct effect of cold may be superimposed on the vasoconstrictor reflex. However, in many instances there is not enough time for the direct effect of cold to account for the intensity of the constriction. Hence, the reflex effects appear to be dominant in the initial constriction. The direct effects of cold develop more slowly as illustrated in the reactions in the forehead and in cases of hypersensitivity to cold.

The vasoconstrictor tone may continue at a high but variable level in the control fingers throughout the cold application. This is probably a factor in maintaining constriction in the cold finger, for relaxation in the control fingers often precedes and accompanies the reactive dilatation in the cold finger.

2. It is the reactive dilatation in the chilled finger which is difficult to explain. The following facts must be kept in mind in examining the mechanism of its production:

a. The reactive dilatation is definitely not due to vasomotor paralysis for it occurs when the vasoconstrictors can be shown to be effective in the chilled finger. However, decreased vasoconstrictor tone in the control fingers may precede or accompany the reactive dilatation in the experimental finger. This

statement does not deny the occurrence of vasoconstrictor paralysis in the chilled finger. Abundant evidence was obtained to show such paralysis during the dilatation and also during the latter part of the period of constriction induced by the cold. But the paralysis is not essential to the reactive dilatation.

b. The reactive dilatation ends either abruptly due to a powerful vasoconstrictor discharge to the fingers or gradually and progressively. The latter is usually the case when vasoconstrictor paralysis continues throughout the period of dilatation. In either case, the resulting constriction may be as prolonged as the first one in response to the cold. The cycle of constriction and dilatation may then recur repeatedly.

c. The reactive dilatation occurs at the end of the cold application during the recovery period despite a depressed circulation in the control fingers and despite vasoconstrictor reflexes being effective in the experimental finger.

For these reasons we consider that the reactive dilatation develops independently of the vasomotor system. Nevertheless, it is subject, in those cases where the vasoconstrictor fibers are not paralyzed by the cold, to modification by the vasomotor system. This position is essentially the same as that of Lewis (1). It has been arrived at by observational techniques permitting a more detailed inspection of the phenomena.

The data presented above do not indicate which components of the finger's vascular system are involved in the constriction to cold and the subsequent reactive dilatation. Analysis of this problem has compelled an extension of the experiments described here and the description of the data in a following paper.

SUMMARY

The vascular reactions in the finger to chilling have been examined by means of the photoelectric plethysmograph. Analysis of these reactions was concerned with the role of the vasomotor reflexes.

The initial immediate constriction on application of cold is due to vasoconstrictor reflexes on which is superimposed somewhat later the direct constrictor action of cold. Evidence:

1. Accompanying constriction occurs also in the warm control fingers of the same and opposite hands, but the constriction is usually more intense in the chilled finger.

2. If a vasoconstrictor reflex is not elicited in the control fingers by an application of moderate cold, the constriction in the chilled finger occurs in a gradual progressive manner, as in the forehead skin, due to the direct constrictor effect of cold on the vessels.

The reactive dilatation, which follows in the chilled finger within three to eight minutes after the application of cold, occurs independently of the vasomotor system. Evidence:

1. The dilatation may be limited to the chilled finger and may occur there when the vasoconstrictor tone is high in the control fingers.

2. Vasoconstrictor reflexes were elicited in the chilled finger during the reac-

tive dilatation in some experiments, while in other instances definite evidence of vasoconstrictor paralysis in the chilled finger was obtained.

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THE REACTIONS OF THE DIGITAL ARTERY AND MINUTE PAD ARTERIES TO LOCAL COLD[1]

ALRICK B. HERTZMAN AND LAURENCE W. ROTH

From the Department of Physiology, St. Louis University School of Medicine, St. Louis, Missouri

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A. *The selective effects of cold on the digital artery and minute pad arteries.* There are several lines of evidence which suggest that the effects of cold on the finger's circulation are selective with respect to the portions of the finger's arterial tree involved in the reactions. First, it has been shown that the larger arteries of the hand and finger (dorsal metacarpal arteries and digital arteries) and also the radial artery, do not ordinarily participate in the vasoconstrictor reflexes which are elicited by such stimuli as startle, a deep breath, or immersion of the opposite hand in ice water, although these stimuli produce marked constrictions in the terminal arteries of the pad (1, 2). These results were interpreted to mean that the vasoconstrictor discharges were selective with respect to those portions of the arterial tree in the hand which were excited to contract.

Second, Lewis (3) developed a strong argument from indirect evidence that the circulatory deficiency in Raynaud's disease resulted from a spasm of the digital arteries and that it was not primarily due to constriction of the terminal vessels. This position implies a specific selective action of cold on the digital artery trunks of these patients, an interpretation which is compatible with the selective character of the vasoconstrictor discharges to the patient's arterial circulation.

Third, Grant's direct observations on the vessels of the rabbit's ear (4, 5) showed that the entire arterial tree of the ear constricted when cold was applied, the maximal effect occurring in the terminal arteries and arterioles. The selective action of cold in his experiments was exerted on the arterio-venous anastomoses and was shown in the reactive dilatation when the arterio-venous anastomoses were the first vessels to open, the central artery of the ear remaining constricted until the rise in temperature caused these vessels to relax again. It was inferred from these experiments that similar reactions occurred in the human finger. The finger's temperature curves during the application of cold and the presence of arterio-venous anastomoses in the finger skin suggested that these vessels were the ones which were responsible for the reactive dilatation in the finger as well as in the rabbit's ear (6). This need not be the case, however. It is quite possible that the reactive dilatation occurs also in the minute arteries and arterioles of the pad and not only in the arterio-venous anastomoses.

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The experiments described below are concerned with the following questions: 1. What is the relative participation of the terminal pad vessels and of the digital artery trunks in the vasoconstrictor reflexes elicited in the chilled finger? 2. Are there differences in the direct effects of cold on the digital artery and the terminal pad vessels? 3. Do the digital artery trunks participate in the reactive dilatation?

METHODS. The experiments have been carried out with the same cooling methods which were described in the preceding paper (7). Records of the volume pulses of the pad and of the digital artery (2) were taken with the photoelectric plethysmograph. Considerable difficulty was experienced in obtaining valid records of the digital artery volume pulses over long periods of time. Slight shifts in the position of the finger will alter the recorded amplitude of the digital artery pulses. These shifts are difficult to prevent. Immobilization of the finger with sponge rubber pads which were cut to support the finger in a fixed position was partially successful in maintaining a constant relation between the digital artery and the plethysmograph. Numbness of the finger during the application of cold prevents the subject from controlling its position. These sources of error probably invalidate the comparison of the amplitudes of the digital artery pulses during recovery and control periods but do not seem to prevent comparison of digital artery and pad artery behaviour during the application of cold.

The amplitudes of the pulses in the pad and digital arteries are considered in these experiments as reflecting corresponding changes in the state of constriction or of dilatation of the corresponding vessels. It is appreciated that the considerable changes in the finger's arterial dynamics in these experiments may modify this relationship. It is also possible that the approximately quantitative relationship shown to exist between the finger volume pulses and the finger blood flow (8) may not hold, particularly during the reactive dilatation, if arteriovenous anastomoses are principally responsible for this reaction.

The temperature of the skin surface as recorded by thermocouples in these experiments probably show only the directional changes in the temperature of the deeper tissues No attempt was made to correlate in a quantitative manner the arterial reactions with the changes in the temperature of the skin surface. Nevertheless, useful qualitative correlations became apparent.

RESULTS. The effects of cold application on the finger temperature varied with the method used to chill the finger and with the state of the circulation in the subjects finger. The rapidity, extent, and duration of the fall in the finger's temperature determined the resultant circulatory effects with respect to the parts of the arterial system of the finger which participated in the reactions and also the extent of their participation.

The relationship of the arterial reactions in the finger to the fall in finger temperature is illustrated in the sequence of figures 1 to 4. With only a slight fall in finger temperature (fig. 1), there is an increase in vasomotor activity (elicited by the cold stimulus) which shows in increased frequency of "spontaneous waves" and reduction in the average amplitude of the pulses in the

pad vessels but which is without effect on the amplitudes of the digital artery pulses. This absence of a reflex vasoconstrictor effect on the digital artery is in

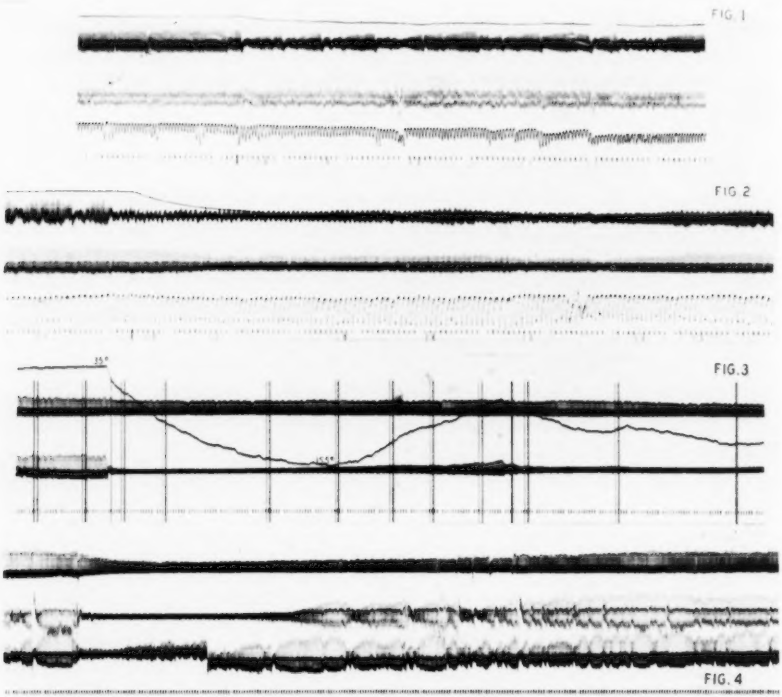

Fig. 1. Reactions of finger arteries to cold which was applied to the whole finger by means of a specially designed copper applicator. Cold applied at arrow and continued for the remainder of the record. Records from above, downwards: finger pad temperature, pad volume pulse, digital artery volume pulse, respiration, time in intervals of five seconds. May.

Fig. 2. Cold by copper applicator to finger at arrow and continued for remainder of record. Top record of finger pad temperature, next of pad volume pulse, next of digital artery volume pulse, respiration, and time in intervals of five seconds. June.

Fig. 3. Cold applied at arrow by copper applicator and continued to end of record. Upper volume pulse record from digital artery, lower from pad. Temperature record from pad. Time in intervals of five seconds. Vertical signals indicate moments of sampling of wave forms on fast kymograph. November.

Fig. 4. Finger chilled by chopped ice at first signal. Ice washed out by warm water at second signal. Upper volume pulse record from digital artery, middle volume pulse record from pad of chilled finger; lower record from control finger of opposite hand. Time in intervals of five seconds. December.

agreement with the previously reported results of other stimuli. When the fall in finger temperature is greater (fig. 2—in this case from 33°C. to 20°C.), the constriction in the pad is more intense and more prolonged and a moderate

constriction appears in the digital artery. The constriction in the latter does not appear at once as is true for the reflex vasoconstriction in the pad, but develops slowly as the finger temperature falls. The gradual character of the constriction of the digital artery would seem to negate its origin in vasomotor reflexes. The digital artery record also shows slow "tonus" waves which are more or less synchronous with the waves in finger temperature. These waves may appear when the pad vessels remain constricted and the finger temperature is moderately below normal levels. Although the evidence is inconclusive, we are inclined to believe that these waves do not have a vasomotor origin, but that they are due to the direct effects of finger temperature on the digital artery.

Chilling with chopped ice (fig. 3) causes a more rapid and a greater fall in finger temperature and induces more marked circulatory reactions: an immediate and profound constriction of the pad vessels (and also those of the opposite hand) and a progressive but fairly rapid constriction of the digital artery. The more rapid constriction of the latter in this instance is probably related to the greater rate of fall in finger temperature with this type of cooling. The extent of the constriction is also usually greater with more complete cooling.

Although the intensity of the sensation of cold and of cold pain depends on the degree of cooling, it seems improbable that vasomotor reflexes elicited by the cold are responsible for the constriction of the digital artery. Thus, in figure 3, the constriction in the control finger is almost maximal before there has been any appreciable reduction in the amplitude of the digital pulse. Further, the gradual character of the constriction in contrast to the abruptness of the pad constriction argues against its vasomotor origin. This point is supported also by the observation that suitable chilling may induce an intense prolonged constriction in the pad without measurable effect on the digital artery pulses (fig. 4). It is probable that, in these instances, the cooling of the digital artery is inadequate to induce its constriction, and yet, that the cold sensation is sufficiently intense to cause a prolonged discharge to the pad vessels.

The reactive dilatation which occurs sooner or later during the continued application of cold is limited to the pad vessels (figs. 3 and 4). As this dilatation warms the finger, it results also in the relaxation of the digital artery. The two dilatations, the one in the pad, the other in the digital artery, may occur so nearly simultaneously that the dependence of the latter on the rise in temperature in the finger produced by the reactive dilatation in the pad may be obscured.

Occasionally, the digital artery may dilate before the pad vessels relax. It is uncertain whether this is to be considered a reactive dilatation or not. Such cases may show a high vasomotor tone in the pad during the entire observation so that the dilatation of the digital artery cannot be due to disappearance of vasomotor tone but must be due to other factors of which the finger temperature is very likely the most important.

Discussion. These experiments show:

First, the digital artery does not participate in the initial constriction which is elicited in the pad vessels by the application of cold to the finger. This constriction has been shown previously to be due to vasoconstrictor reflexes, the

constriction possibly being augmented by the direct effects of cold on the pad vessels (7). Failure of the digital artery to constrict at this moment is in line with previously reported data showing the usual absence of vasoconstrictor reflexes in the digital artery (2). Since the vasoconstrictor discharge to the finger may be intense and prolonged, its failure to reach the digital artery implies either that this vessel does not have a vasoconstrictor supply or that the vasoconstrictor discharge is selective, reaching only the minute arteries and arterioles. Anatomical information refutes the first alternative, but it is worth emphasizing that we have not seen a convincing illustration of a vasoconstrictor reflex in the digital artery. We are inclined, however, to interpret these experiments in terms of a selective vasoconstrictor discharge.

Second, the digital artery is constricted by the direct effects of cold on the vessel. This is probably also true of the pad vessels but the effects here are obscured by the vasoconstrictor reflexes which are acting simultaneously. The extent of the constriction of the digital artery seems to be roughly proportional to the fall in finger temperature. Exact proportionality cannot be inferred from measurements of the temperature of the skin surface.

Third, the reactive dilatation which appears during the application of cold is probably limited to the pad vessels. Apparent participation of the digital artery seems to be due to the rise in temperature which results from the reactive dilatation. This indicates that the mechanism of the phenomenon is located in the minute vessels. Either the liberation of a dilator substance acting through an axone reflex on the minute arteries and arterioles as suggested by Lewis (9) or a protective dilatation of arterio-venous anastomoses as suggested by Grant (6) would be compatible with our data. It has not yet been possible to separate the relative participation of small arteries, arterioles, and arterio-venous anastomoses in the reactive dilatation in the finger. However, it seems improbable that this reaction is limited to the arterio-venous anastomoses since the amplitudes of the pad pulses at the height of the reactive dilatation would imply a wide dilatation also of the small arteries and arterioles of the pad.

It is interesting to speculate on the significance of the subject's sensations during the reactive dilatation. The throbbing is experienced with the opening of the pad vessels. It is definitely not correlated with dilatation of the digital artery. It is therefore produced by the minute vessels and may be experienced before the reactive dilatation has resulted in a pad pulse equal in size to the control level. As this reaction increases and the pad pulses become still larger, throbbing tends to disappear. Does this mean that throbbing results from the pulse entering channels which are usually closed, e.g., the arterio-venous anastomoses?

B. *The effects of cold on the propagation of the pulse in the finger arteries.* During the experiments on the effects of cold on the finger's arterial circulation, we observed that, in a few apparently normal subjects, the usually peaked form of the pad pulse may be altered to the plateau type during the reactive dilatation. This change occurred at the time when the blood flow was increasing rapidly. The similarity in the form of the pad pulse during the reactive dilatation to that

observed in resting hypertensive patients (10), suggested that we had inadvertently produced by the action of local cold in normal subjects the same changes in the arterial dynamics of the finger as exist in the hypertensive patient. The selective character of the effects of cold on the large and small arteries of the finger increased the probability of this suggestion.

METHOD. Analysis of the wave forms of the volume pulses of the finger pad was effected by shunting high frequency galvanometers across the recording milliammeters when the previously recorded experiments on cold were performed (7) and then recording the pulses at suitable moments on a separate high-speed photo-kymograph. Recording of the wave forms was so selected at the time of the experiment that representative samples of the volume pulse waves were secured during dilatation and constriction in the control, experimental, and recovery periods.

Analysis gave attention to the form of the wave and the crest times, following the procedure as previously reported (10). The crest times of the pad volume pulses depend on the time required for the minute pad vessels to reach peak filling during an individual pulse cycle. The crest times are therefore affected by the relative constriction of the digital artery and of the minute arteries of the pad. Selective constriction of the latter may be expected to shorten the crest time of the pad pulse. Selective dilatation of the pad vessels in the reactive dilatation due to cold may, on the other hand, increase considerably the crest time due to the larger capacity of these vessels and the possibly slower arrival of the pulse wave through the partially constricted digital artery. It will be seen that these effects on the crest times are not measurable unless the changes in the lumens of the digital artery and of the pad vessels are considerable. Moderate changes as in mild vasomotor reflexes do not measurably influence the propagation of the pulse in the finger arteries (10).

RESULTS. In the usual experiment (fig. 5 and table 1) the effects of cold on the propagation of the pulse were minor; there was little change in the form of the pad pulse. During the onset of the initial constriction (with the application of cold), crest times were shortened in both control and experimental fingers but more so in the latter in which the constriction was also greater. As constriction continued, the crest times were lengthened towards the values of the control period. The dicrotic notch was placed higher on the catacrotic limb during the constriction. As the reactive dilatation began, the crest times were lengthened slightly, returning to normal values when the vessels were well dilated. The dicrotic notch was usually placed higher at the beginning of the reactive dilatation, falling again as the latter developed. The pulse waves had the usual peaked form during the constriction but tended to be very slightly rounded during the reactive dilatation. In general, then, the effects of the constriction to cold and of the subsequent dilatation, on the form of the finger pulses, were very moderate.

However, in several apparently normal subjects, there occurred reversible changes in the wave forms which resembled those seen in resting hypertensive and arteriosclerotic subjects (10). We present two examples. In the experiment

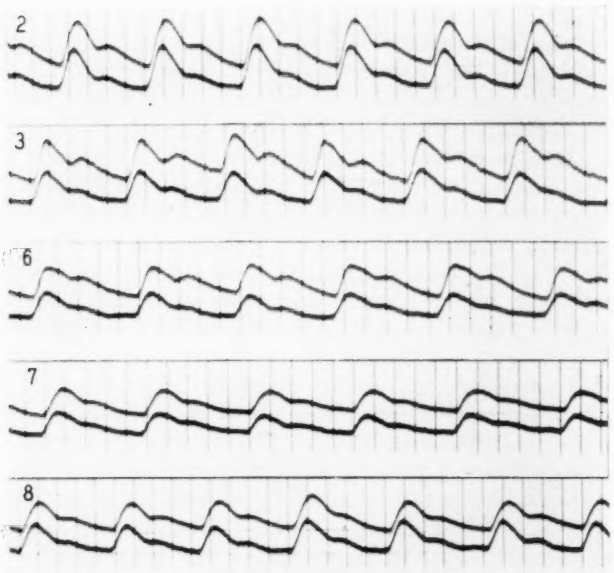

Fig. 5. Records of volume pulses by high frequency galvanometers taken at vertical signals in figure 1 of preceding paper (7). Records 2, 3, 6, 7, 8 were taken at the moments numbered correspondingly in that figure. Records 3 and 6 of the chilled finger's pad pulses were taken with increased amplification. Upper wave, chilled finger; lower wave, control finger. Time: 0.15 sec.

TABLE 1

Crest times and position of dicrotic notch on finger pad volume pulses during local application of cold to the finger

RECORD	CREST TIMES IN SECONDS		ELEVATION OF DICROTIC NOTCH IN % OF WAVE		PERIOD OF EXPERIMENT
	Control finger	Chilled finger	Control finger	Chilled finger	
2	0.12	0.13	41	50	Control
3	0.11	0.12	47	65	Control—during slight constriction
4	0.08	0.06	40	74	Initial constriction to cold
5	0.13	0.14	40	68	Early in reaction
6	0.13	0.13	45	61	Later in reaction
7	0.09	0.07	53	57	During marked constriction. Cold
8	0.10	0.11	52	61	Later in constriction. Cold

Record numbers correspond to those in figure 5 of this paper and in figure 1 of the preceding paper (7).

of figure 6, we note no unusual effects on the time relations. But the tendency towards a plateau in the pulse of the chilled finger is exhibited during the reactive dilatation when the pulse of the control finger is peaked and when the volume

pulse amplitudes are the same in the two fingers (records 7, 13, 16 of fig. 6). The plateau is due to elevation of the dicrotic notch and the "zwischenschlag". The latter is a small wave between the crest of the pulse and the dicrotic notch. There is a faint suggestion of it in the control record 2. It is very obvious in the pulses of the control and chilled fingers in records 13, 16 and 17. It is the crest of the wave of the chilled finger in the first, second, third, and fifth pulses of rec-

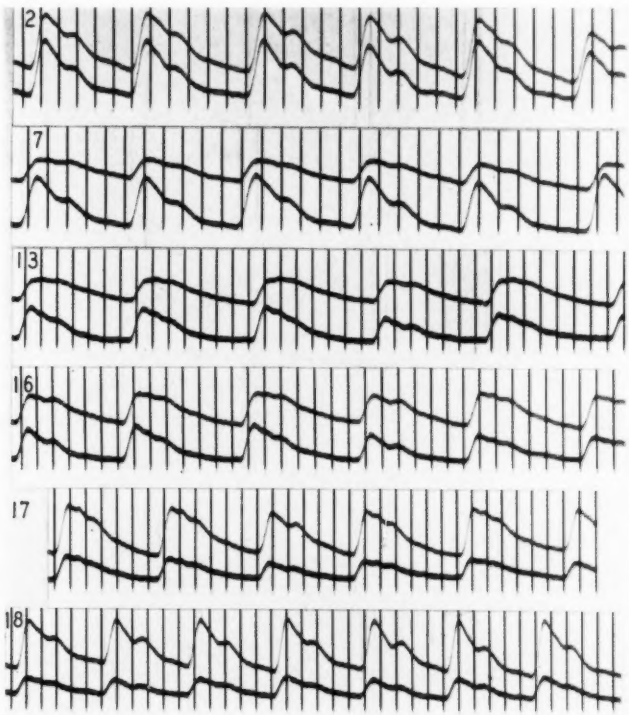

Fig. 6. Records of volume pulses of same subject as in figure 4 of preceding paper (7), but from a different experiment. Record 2, during control period. Record 7, during well developed reactive dilatation (control finger temperature 35°C.; chilled finger temperature 16°C.). Record 13, during the reactive dilatation (finger temperatures 34.6°C. and 14°C.). Records 16, 17, 18, during early part of recovery; record 16, 2 minutes after application of cold was stopped (temperatures 35°C. and 15°C.); record 17, 2 minutes later (temperatures 35°C. and 28°C.); record 18, 6 minutes later (temperatures 35°C. and 34°C.). Upper wave: chilled finger; lower wave; control finger. Time 0.15 sec.

ord 13. If the crest time were considered as the time to the "zwischenschlag", this would give a considerable increase in the value as was found to be the case in hypertension and arteriosclerosis (10). It was suggested then that ascent of the "zwischenschlag" was responsible for the increase in crest time. We have here an example of the experimental reversible production of a similar effect in a subject with apparently normal arteries.

The experiment of figure 7 was done somewhat differently on an older subject in good health. The finger was immersed in chopped ice and the records taken after the finger had been withdrawn from the ice and while it was exposed to room air. This procedure is most effective in securing marked constriction of the digital artery. Record 2 was obtained immediately on withdrawal from the ice

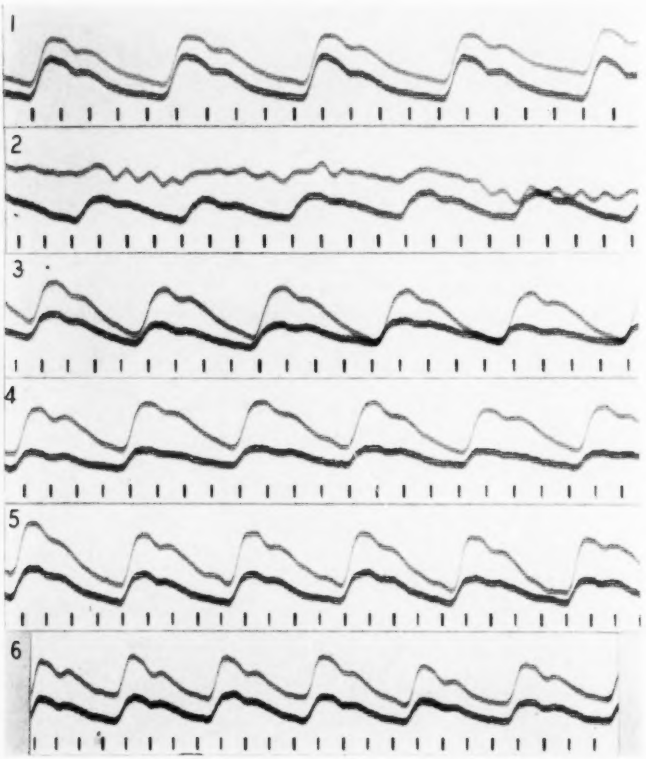

Fig. 7. Effects of ice-water on finger volume pulses. Subject: male 42 years old. Normal blood pressure. Record 1, during control period. Record 2, immediately following immersion in ice water for 2 minutes—finger cold, aching, burning. Record 3, immediately following immersion for 6 minutes. Finger warm, throbbing. Record 4, 1 minute later. Record 5, 2 minutes after record 3. Record 6, 4 minutes after record 3. Upper wave: chilled (R2). Lower wave: control finger (R4). Time 0.2 sec.

while the finger was constricted. Records 3 to 6 were taken in another run during the reactive dilatation which began while the finger was still in the ice. The control record, number one, shows a peaked wave with a slight tendency towards a plateau due to high location of the dicrotic notch and the "zwischenschlag"—the latter is barely discernible. The crest times are normal in this control record. During the reactive dilatation, the "zwischenschlag" ascends

to become the crest of the wave. This increases the crest time to the values found in hypertensive and arteriosclerotic subjects.

INTERPRETATION. Theoretical considerations suggest the following possibilities in the effects of the constriction to local cold and of the subsequent reactive dilatation on the propagation and form of volume pulses in the finger:

1. Constriction of the pad arteries without much change in the digital artery trunks should increase digital artery pressures somewhat with resultant slightly faster propagation of the pulse and also decrease the time required for the pad vessels to reach peak filling during the pulse cycle. The crest times should therefore be shortened. This happens regularly during the early part of the initial constriction to cold. We have shown above that the digital artery trunks do not participate significantly in the constriction at this time. This fact is in agreement with the shortening of the pad crest time during the initial constriction to cold.

2. Constriction of the digital artery trunks as well as of the pad arteries later during the application of cold would tend to offset the effects of constriction in the pad arteries on the crest times. These should increase slightly, as they do, a little later in the initial constriction, due to slower propagation of the pulse.

3. Continued considerable constriction of the digital artery trunks with progressive relaxation of the minute pad arteries (possibly the arterio-venous anastomoses and arterio-venous shunts) would slow the propagation of the pulse, increase the time for peak filling of the minute vessels during the pulse cycle, and so increase the crest time. This is the case early during the reactive dilatation which is limited to the pad vessels and which does not affect the digital artery trunk until the rise in finger temperature causes relaxation of this vessel. Quantitative differences between individuals may depend on the relative effects of the degree of constriction of the digital trunks and of the pad arteries on the propagation of the pulse wave. This situation is analogous to that described by Grant (4) in the rabbit's ear in which the main artery trunks of the chilled ear remained partly constricted until the opening of the arterio-venous anastomoses permitted a larger flow of blood with a rise in the ear temperature.

4. When dilatation of the minute pad vessels (arterio-venous anastomoses, etc.) is followed by dilatation of the digital artery trunk later in the reactive dilatation, it should result in a decrease of the crest time to normal values. This is the case in the later stages of the reactive dilatation.

This interpretation of the changes in the form and crest times of the pad pulses during the application of cold is in line with the direct demonstration (above) of the selective effects of cold on the finger's arterial circulation. It may be noted, however, that this theoretical interpretation was formulated from the pad pulse records before the experiments on the digital artery were carried out.

Evaluation of the meaning of the changes in wave form illustrated in figures 6 and 7 is a more difficult problem. Ordinarily, a plateau form of the peripheral arterial pulse is associated with an increased peripheral resistance and an altered elasticity of the vessels. In these two experiments, the plateau form appears also at the time when the vessels are dilated, when the flow is high and the finger is

throbbing. At the same time, the pulse in the neighbouring control finger may be peaked (fig. 6), so that the plateau in the pulse in the chilled finger must be due principally to the local arterial dynamics in the chilled finger. The effects of selective changes in tone of the digital artery trunks, of the pad arteries and arterioles, and of the arterio-venous anastomoses, on the reflection and damping of pulse waves, are pertinent to interpretation but must be considered a purely speculative matter. However since the plateau form disappears when the reactive dilatation is well advanced (record 18 of fig. 6) and the digital artery trunk is probably dilated, it seems possible that the plateau form develops when the arterio-venous anastomoses and other small arteries are widely dilated and the larger arteries are still constricted. As the finger warms, the arterio-venous anastomoses probably close, the larger arteries are dilated again and a rapid flow occurs through the usual channels. This reëstablishes the usual arterial dynamics in the finger. The changes illustrated in figure 6 fit this concept which is supported by the experiments described above as well as by the direct observations of Grant on the rabbit's ear (4).

We may then ask, why does not plateau formation appear in the usual experiment? In a sense, it does, as the dicrotic notch is elevated (higher than in the control) in nearly all instances during the early part of the reactive dilatation. Differences in the effects of the latter on the wave form of normal subjects may then be due to quantitative differences in the degree of participation of the components of the finger's arterial system and are not necessarily due to qualitative differences in behavior.

The striking similarity in the effects of hypertension and of cold in some normal subjects on the propagation of the pulse in the finger arteries poses some interesting problems. It has been argued from measurements of brachial-digital pressure gradients that the increase in resistance in hypertension is located in the small arteries and arterioles of the finger (11). If this is all that happened, the crest times of the pad pulses should be shortened, as is the effect of pad constriction in the normal subject. But the reverse has been observed without exception in every case of hypertension studied (10, and unpublished data). If there were an increase in the resistance in the digital artery and of other large hand arteries in hypertension (which is denied by the brachial-digital pressure gradients) this would lengthen the pad crest time, but the constriction of the digital artery in the normal subject does not increase the pad crest time to the values observed in hypertension. This does not necessarily eliminate the digital artery and other large hand arteries from modifying the pulse in its propagation into the minute vessels of the finger pad. This possibility is being studied.

There remains the possibility in hypertensive cases that blood is shunted along such channels as the arterio-venous anastomoses, as may also be the case during the reactive dilatation in normal subjects. The extent to which these vessels can affect the finger pulses and blood flow is unknown. However, if their opening occurs at a time when the small arteries and arterioles are constricted (as in the hypertensive patient and during cold in the normal subject)

and if their dilatation can restore blood flow in the finger, the form of the pad volume pulses may well differ from the normal. It is therefore extremely interesting that, in some normal subjects, the reactive dilatation to cold (when the arterio-venous anastomoses presumably open) results in a pad volume pulse which is similar to that seen in hypertensive patients.

SUMMARY

A. The selective effects of local cold on the terminal pad vessels and the digital artery of the chilled finger were demonstrated by means of photoelectric plethysmographs.

The digital artery does not participate in the vasoconstrictor reflexes elicited by the cold. Its later constriction during the continued application of cold appears to be due to the direct effects of the fall in temperature on the artery.

The reactive dilatation which appears during the application of cold is limited to the minute pad vessels and does not involve the digital artery until the resultant rise in finger temperature permits relaxation of this artery.

B. The effects of these reactions on the propagation of the pulse in the finger's arterial system were studied by recording the pad pulses with high frequency galvanometers.

In the usual experiment, the time relations and form of the pad pulses in the chilled finger were altered only moderately and in the direction which could be predicted from the relative participation of the pad and digital arteries in the reactions to cold.

In a few normal subjects, the reactive dilatation produced a pad pulse similar to that seen in chronic hypertension, thus suggesting that one of the factors responsible for the change in pad pulse form in hypertension may be the shunting of blood through direct arterio-venous communications.

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THE ABSENCE OF VASOCONSTRICTOR REFLEXES IN THE FOREHEAD CIRCULATION. EFFECTS OF COLD[1]

ALRICK B. HERTZMAN AND LAURENCE W. ROTH

From the Department of Physiology, St. Louis University School of Medicine, St. Louis, Missouri

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Plethysmographic data were presented in earlier publications (1, 2, 3) to illustrate the selective character of vasomotor activity in the skin. These plethysmograms indicated the absence of vasoconstrictor reflexes in head skin (with the exception of the ear). It has been suggested that these results "may be due rather to relative vasomotor inactivity of certain vessels than to an independence of rhythmic activity" (4).

It is possible that vasoconstrictor fibers do not reach the forehead skin. There are many observations which so suggest: the constancy of the forehead temperature and its failure to fall during prolonged constriction of the fingers and toes; the throbbing of the temporal artery during excitement; the frequent increase in forehead volume pulses during vasoconstrictor discharges (1); the relative constancy of the blood supply of head skin during massive disturbances of the circulation by drugs (5); the failure of unilateral cervical sympathectomy to increase the temperature of the forehead skin on that side (6)—the effects are also very small on the cheek, upper lip, nose and chin.

The present communication reports data which extend this list and further deny the existence of vasoconstrictor reflexes in the forehead skin. The reactions of the minute arteries in the forehead skin were studied by recording their volume pulses with the photoelectric technique which has been described elsewhere (7).

RESULTS. Spontaneous arterial constrictions which are seen so commonly in plethysmograms of the extremities and which are generally considered as due to vasomotor activity, have not been observed in the forehead skin. A striking illustration is provided in figure 1 obtained from a normal male while he rested, slept, and awakened. The frequent vasomotor discharges to the finger are wholly absent in the forehead. Reduction in finger flow (third record), probably due to increased vasomotor tone, occurred without evident change in the forehead circulation.

Startle due to loud unexpected noises, immersion of the hand in ice water, or a deep breath, are known to elicit vasoconstrictor reflexes which not only affect both the upper and lower extremity, but also are often sufficiently extensive to increase the arterial blood pressure. If these stimuli are effective, the finger's

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vessels are always constricted in the normal subject. At times, the vasoconstrictor reflexes so elicited may include the vascular areas in the calf and forearm (8), but the constrictor effects here are weak compared to those in the hand and foot. We have seen no instance of arterial constriction in the forehead in response to these stimuli, irrespective of how intense the effects may have been in the finger. Figure 2 illustrates. The stimuli of a loud noise, of immersion of the opposite hand in ice water and of a deep breath were supplied in succession. The effect of awakening the sleeping subject by the observers' cough is also

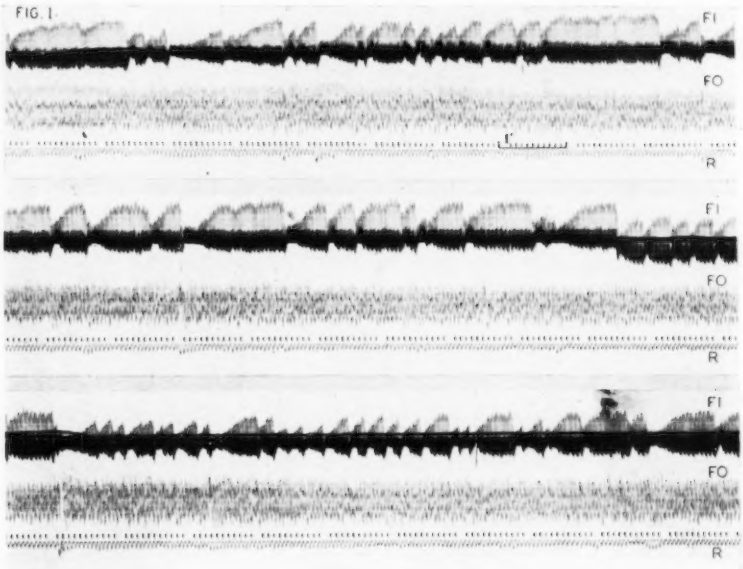


Fig. 1. Effects of spontaneous vasomotor activity on the finger and forehead vessels in the resting subject who was asleep and snoring during most of the record, which is continuous. Upper volume pulse record from finger, lower from forehead. Respiration. Time: 5 seconds. Records of temperatures of finger and forehead taken by thermocouples do not show in the reduction of the figure but show no large changes in the original. Room temperature varied between 23°C. and 25°C.

shown. Decisive constriction occurred in the finger in each case. If there had been a weak constriction in the forehead skin, there would have been at least a slight decrease in the volume pulse here. But there is no indication whatever in these records of a vasoconstrictor action on the forehead arteries.

An effective method of eliciting constriction in cutaneous vessels is the local application of cold. Effective chilling of the forehead skin in such a manner as to permit simultaneous and continuous recording of the volume pulses of the chilled skin proved to be fairly difficult. We tried various applicators made of rubber dam through which cold water could be circulated. These were large enough to cover most of the forehead. A hole cut in the center permitted the

plethysmograph to reach the small skin area which was surrounded by skin in contact with the cold surface of the applicator. Dissatisfied with the small fall in temperature so induced in the skin directly beneath the plethysmograph,

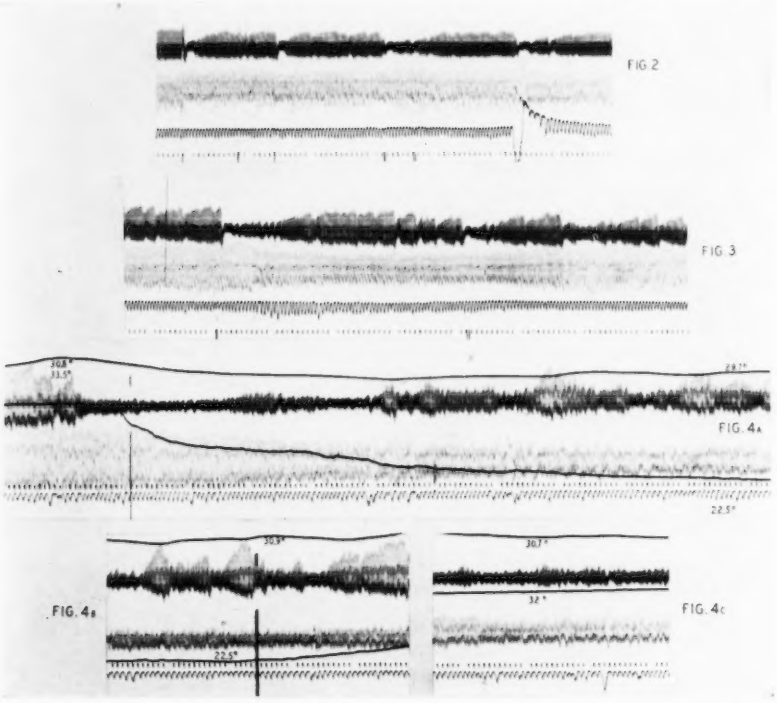


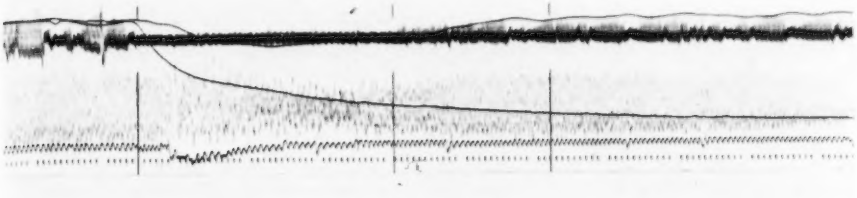
Fig. 2. Absence of vasoconstrictor reflexes in forehead skin. At *1*, the sleeping subject was awakened by the observer's cough. At *2*, a loud noise. At *3*, immersion of the opposite hand in ice water. At *4*, a deep breath. Upper volume pulse record from finger pad, lower from forehead. Respiration. Time: 5 sec.

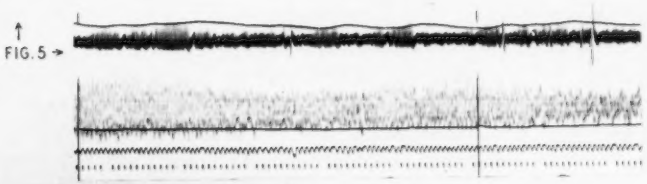
Fig. 3. Effects of moderate cold applied to the forehead by means of cold water circulated through a hollow rubber applicator which was in contact with the forehead. The cold was applied between the vertical signals. Upper volume pulse record from the finger pad, lower from the forehead. Respiration. Time: 5 sec.

Fig. 4. Effects of more prolonged application of more intense cold than that applied in figure 3. The cold was applied at the break in the forehead temperature curve. Recovery of the forehead circulation was incomplete at the end of the observational period. No pain was experienced. The time intervals between the sections of the figure are: between sections 1 and 2, 12 minutes: between sections 2 and 3, 21.5 minutes. The records from above, downwards: finger pad temperature, finger pad volume pulse, forehead temperature, forehead volume pulse, time (5 sec.), respiration.

we constructed an applicator of segments of copper sheeting which were soldered to short pieces of copper tubing. The latter were connected closely together by rubber tubing. This arrangement provided an excellent broad comfortable

contact with the forehead skin. A gap in its middle provided for the application of the plethysmograph, as with the rubber applicator. Despite the excellent contact with the skin and the high thermal conductivity of copper, this device was not much more successful than the rubber applicator in lowering the temperature of the skin either directly under it or under the plethysmograph. The





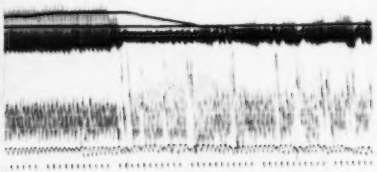


Fig. 5. Relation of the effects of cold on the forehead circulation to the sensations experienced. The cold was applied to the forehead by means of a special copper applicator, at the first vertical signal. At the second signal, there was intense pain which was localized under the applicator. At the third signal, the pain was somewhat less intense. At the fourth signal, the pain was described as moderate. At the fifth signal, the pain has disappeared. Records from above, downwards: finger pad and forehead temperatures, finger pad volume pulse, forehead volume pulse, respiration, time (5 sec.).

Fig. 6. Temporary increase in the forehead volume pulse on the application of cold to the forehead. "Quite intense pain for the first minute or so" was reported by the subject. The cold was applied just preceding the constriction in the finger. The artifacts in the forehead record were due to frequent swallowing movements. Upper volume pulse record from the finger pad; lower from the forehead. Respiration. Time: 5 sec.

cold applicator and the plethysmograph were suspended from a plaster of Paris cast of the subject's cranium as illustrated elsewhere (9). This device is comfortable and permits prolonged observations.

On starting the flow of cold water (chilled to 4–8°C.) through the applicator, constriction occurred at once in the control finger but not immediately in the forehead skin which was being cooled (figs. 3–6). The intensity and duration of the constriction in the finger varied with subjects; there was no obvious correla-

tion with the sensations. Constriction appeared gradually in the forehead skin as its temperature fell. Cooling took place rather slowly and its action on the forehead vessels required periods as long as twenty minutes to attain a maximal decrease in the volume pulse. The cooling was insufficient in all instances to secure complete constriction. The decrease in pulse amplitude varied in the individual experiments between 32 and 90 per cent of the control value. In no instance was there any indication of a vasoconstrictor reflex in the forehead skin, although in many trials the reduced volume pulses and the increased frequency of "spontaneous waves" in the finger during the cold application showed that the cold stimulus continued to be effective in eliciting vasoconstrictor reflexes.

The absence of the initial constriction to cold in the forehead skin is comparable to those rare cases described in a previous paper (10), in which chilling of the finger or toe results in *gradual* constriction when vasoconstrictor reflexes are *not* elicited by the cold. Also, the sympathectomized toe responds to cold as does the forehead skin (10). These analogies in responses strongly support our thesis that vasoconstrictor reflexes are wholly absent from the forehead skin.

In a few experiments (figs. 5 and 6), the forehead volume pulse was considerably increased during the cold application at the time of sharp constriction in the finger (fig. 6) or when pain in the forehead was experienced (fig. 5). This effect may have been due to an increase in pulse pressure or to a vasodilator reflex elicited in the forehead by the pain. There is evidence that vasodilator fibers reach the forehead by way of the cervical sympathetic (6). Thus, neither emotional flushing nor the flushing due to over-heating occur on the denervated side of the face and forehead after unilateral cervical sympathectomy (6).

The increase in amplitude of the forehead volume pulses (fig. 5) with the onset of pain, when constriction of the forehead vessels due to cold had already occurred, recalls the correlation between the amplitude of the temporal artery pulsations and the presence or absence of headache (11). However, none of the subjects confused the pain from the local cold application with headache. The pain was somewhat similar to a sharply localized headache but contrasted notably with the intense frontal ache produced by strong winds on a bitter cold day. The difference seemed to be primarily one of intensity and may well have been due to failure to cool adequately. Some subjects experienced only a sensation of cold.

The reactive dilatation which is so characteristic of the finger's reactions to cold was not observed in the forehead skin. This may have been due to inadequate cooling as we seldom succeeded in chilling to the point where the constriction was sufficient to eliminate the volume pulse. The lowest temperature reached in these experiments was 21°C. which is above that at which the reactive dilatation is usually induced in the finger. It is also possible that the arteriovenous anastomoses are too few in number in the forehead skin for a reactive dilatation to occur. Information is lacking on this point.

Recovery of the forehead circulation and of the forehead temperature after ending the cold applications proceeded slowly, thus contrasting sharply with the recovery in the finger (10) where the rise in temperature and the arterial dilata-

tion occurred rapidly. The slowness of recovery in the forehead may have been related to the absence of any indication of a reactive dilatation there. It may also have been affected by the thermal capacity of the cold applicator. Yet, recovery in the finger was also subject to the same delaying influence but proceeded swiftly.

SUMMARY

The absence of vasoconstrictor reflexes in the forehead is demonstrated by the following evidence:

1. The spontaneous rhythmic constrictions of vasomotor origin in the finger of the resting subject are absent from the forehead.

2. The vasoconstrictor reflexes elicited by startle, awakening, a deep breath, immersion of the hand in ice water or local cold to the forehead are wholly absent in the latter.

3. The vascular reactions of the forehead skin to local cold are like those of a sympathectomized digit. Constriction is gradual as the temperature of the skin falls and seems to be due to the direct effects of cold on the vessels.

The reactive dilatation to cold which occurs in the fingers, was not observed in the forehead skin.

Occasionally, there was indication of a vasodilator reflex in the forehead skin at the time of a powerful constriction in the finger.

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